THE OHIO JOURNAL OF SCIENCE

VOL. XXXIII SEPTEMBER, 1933 No. 5

FOREWORD.

The Symposium on Metabolism, which is presented here, has as its basis a course of lectures that was given before the Ohio State Chapter of the Society of the Sigma Xi during the academic year 1932–33. At that time the personnel of the Executive Committee, which is charged with determining the policies of the chapter, was as follows:

Professor J. F. Lyman, *President.*
Professor F. C. Blake, *Councilor.*
Professor E. M. Spieker, *Vice-President.*
Professor F. A. Hitchcock, *Secretary.*

It was the unanimous opinion of this committee that the program for the year would be both more valuable and more interesting if it were arranged so that various aspects of the same central topic furnished the subjects for the meetings held throughout the year. After careful consideration the subject of Metabolism was selected as the central topic. While it was recognized that a course of lectures on this topic would be of interest primarily to the biologists of the Chapter, it was believed that the subject involved sufficient applications of physics and chemistry to make the course worth while to the non-biological scientist.

The authors of the various papers have all made valuable contributions to our knowledge of metabolism, and the major portion of the material given in each paper is obtained from the original researches of the author. The material is presented in such a way as to make it comprehensible to the intelligent layman.

The committee wishes to express its appreciation to the authors of the various papers, to the editor and business manager of the Ohio Journal of Science, to the members of the Chapter

and to all others concerned, for the splendid co-operation which made the series of lectures so successful and which now makes their publication possible.

F. A. HITCHCOCK, *Secretary.*

Physiology Laboratories,
Ohio State University, September 8, 1933.

EDITOR'S NOTE.

The Journal is pleased to be able to publish these papers on metabolism, which form an important contribution to science, and which conform admirably to the Journal's policy of printing timely articles on topics of current scientific interest. They are published in symposium form and out of their regular order of appearance due to the financial aid of the Society of the Sigma Xi.

THE DEVELOPMENT OF METHODS FOR DETERMINING BASAL METABOLISM OF MANKIND

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When we breathe, oxygen is absorbed from the air in the lungs and carried by the blood to all parts of the body. The oxygen unites with the protein, fat, and carbohydrate available, produces carbon dioxide and water and in this oxidation process heat is generated. Lavoisier (1), in 1777, discovered that animals confined in an enclosed space absorbed oxygen from the enclosed air and gave off carbon dioxide. Later he and La Place (2) found that the heat given off by a guinea-pig in an ice calorimeter nearly equalled that which would be produced in the same length of time by the oxidation of the carbon equivalent to that given off in the carbon dioxide produced by the guinea-pig. They thus established a relationship between direct and indirect calorimetry. In 1789 Seguin and Lavoisier (3) made their respiration experiments on man without food at two environmental temperatures, thus determining the first basal metabolism values on a human subject. We do not have all the details of the apparatus used, but we know that a face mask was employed and that the expired air was collected and then analyzed for oxygen and carbon dioxide. Since that time various methods have been used to measure the products of combustion in the body, the carbon dioxide excreted or the oxygen absorbed, or directly the heat eliminated. I shall mention some of the methods with respect to various types that have been developed and modified from the time of Lavoisier to the present day. It is impracticable to describe in detail all of the apparatus used, but I hope to show in a general way the gradual development of the methods and apparatus up to the present-day simplifications and refinement.

Allen and Pepys (4), in 1808, described an apparatus in which the subject breathed through a mouthpiece. By means of valves operated by hand, the subject inspired from a gasom-

eter with water as a seal and expired into gasometers with mercury as a seal. Only the carbon-dioxide production was measured.

Andral and Gavarret (5), in 1843, used a copper mask with rubber edges, which could be fitted to the face. This had valves which prevented the subject from expiring into the outside air. A tube was connected to a series of glass globes in which a vacuum had been established before the experiment began. The expired air was collected by suction, and subsequently the carbon dioxide in this air was absorbed and the increase in weight of the absorption train determined.

Smith (6), in 1859, used a metal mask with valves, a meter to measure the inspired air, and containers with sulphuric acid and potash solution to absorb water vapor and carbon dioxide. Figure 2 shows the arrangement of the apparatus. The box container for the potash solution and the sulphuric acid container following it were weighed to obtain the output of carbon dioxide.

C. Speck (7), in 1871, used a pair of gasometers, one for the inspired air and one for the expired air. A nose clip closed the nostrils, and breathing took place through a tube placed in the mouth. Valves were employed to separate inspired and expired air. The changes in the weights of the bells of the gasometers were compensated by decrease and increase in weights in the counterpoises. A sample of expired air was analyzed for both carbon dioxide and oxygen.

Regnard (8), in 1879, collected the expired air in a rubber bag of about 200-liter capacity. The breathing was through a mouthpiece, and the inspired air was separated from the expired air by valves. A sample of the air in the bag was later analyzed. The modern counterpart of the bag method is the wedge-shaped bag devised by Douglas (9) in 1911. This is transportable and may also be used in rest experiments. The chief criticism against the use of rubber for collection of expired air is its permeability to carbon dioxide. This has been recently overcome by Mueller (10), who coated the inside of the bag with tinfoil and then vulcanized it.

In 1887 Geppert (11) reported measurements of the total expired air by a wet meter and had an arrangement for drawing duplicate proportional samples directly into a gas analysis apparatus. The samples were subsequently analyzed for carbon dioxide and oxygen.

Hanriot and Richet (12) in 1891 adopted a principle in the determination of the respiratory exchange that is very simple theoretically. They employed three meters in series. The first meter measured the volume of inspired air, the second meter measured the volume of expired air, and the third the volume of expired air after the carbon dioxide had been absorbed. The difference between the first and third meters gave the volume of oxygen absorbed. A mask and Mueller (water) valves were used for separating inspired and expired air.

Scharling (13), in 1843, had a chamber of 1 cubic meter content suitable for humans. It was ventilated by an air pump or aspirator, and the entire air was conducted through a potash solution to absorb the carbon dioxide. The ingoing air was also freed from carbon dioxide by a potash solution.

Pettenkofer (14), in 1862, constructed a chamber large enough for a man to remain in it 24 hours or more. The chamber was continually ventilated, and the ingoing and the outgoing air were continuously sampled proportionately for carbon dioxide and water vapor. The water vapor was absorbed by sulphuric acid and the carbon dioxide by barium hydroxide, which was afterward titrated. The total ventilation through the apparatus and sampling arrangements was measured by gas meters.

Liebermeister (15), in 1870, constructed a chamber suitable for a man, and of such a shape that the subject could sit or recline. The chamber was ventilated, and the carbon-dioxide production was determined periodically in small samples of the outgoing air by absorbing the carbon dioxide in a barium hydroxide solution and later titrating the solution with oxalic acid. He then constructed a graph of the changes in composition of the outgoing air and by means of a differential equation expressing the relationship between the ventilation, the time elapsed between samples, and the carbon-dioxide content of the outgoing air, he was able to calculate the carbon-dioxide production.

The principle of determining the respiratory exchange with a chamber by conducting air through the chamber and passing samples of the ingoing and outcoming air through absorbers as applied by Pettenkofer and Voit, has been employed by a number of other workers. The early form of the respiratory calorimeter in Middletown, Connecticut, described by Atwater, Woods, and Benedict (16) in 1897 utilized this principle. A

special pump was devised for withdrawing an aliquot of the air leaving the chamber, and this aliquot was subsequently passed through absorbers for water vapor and carbon dioxide. This chamber was large enough for a man to live in for days and weeks at a time.

In 1903 Jaquet (17) described an irregular shaped chamber of such a size that a man could either sit in the apparatus or could recline upon a bed. The outcoming air was measured by means of a gas meter, and an aliquot sample was taken for analysis. This was subsequently analyzed for carbon dioxide and oxygen.

In 1910 (18) Grafe published a description of his respiration chamber for bedridden patients only, the top of which could be raised easily in order to roll in a patient on the bed. In 1909 (19) he also described his head respiration apparatus, an arrangement that covered the head and shoulders of the patient. Both of these apparatus of Grafe were ventilated on the open-circuit principle, and an aliquot sample of the outcoming air was analyzed for carbon dioxide and oxygen.

A chamber of large size that would hold more than one person was constructed by Sondèn and Tigerstedt (20) and described in 1895. Only the carbon-dioxide production was determined with this chamber apparatus. In 1919 Benedict (21) reported the construction of a large group chamber capable of holding at least twelve individuals in bed, by which the carbon dioxide elimination of a group could be determined. The aliquoting of the outgoing air was by a unique principle.

Not only did Lavoisier study the respiratory exchange by the open-circuit principle but also he made some experiments on the respiratory exchange of animals in a closed system. This principle, however, was not carried out with man until a number of years later.

The principle of the closed-circuit system of Regnault and Reiset (22) was applied to man by Hoppe-Seyler (23) in 1894. The carbon dioxide was absorbed from the air by means of a potash solution, and oxygen was admitted to the system through a gas meter. The carbon dioxide was afterwards driven off from the potash solution by means of sulphuric acid and absorbed in an apparatus for carbon dioxide, and the apparatus was weighed. The oxygen used was obtained from the readings of the gas meter and from an analysis of the air in the respiration chamber.

In 1905 (24) the respiration calorimeter at Middletown, Connecticut, was brought to a state where the respiratory exchange of man in a chamber could be determined in short periods of from one to six hours with a high degree of accuracy. Subsequently the general principle and mechanical arrangements used in this apparatus were applied to the construction of respiration calorimeters in the Nutrition Laboratory (25). With the closed-circuit system of both the Middletown and Boston calorimeters the water vapor elimination and the carbon-dioxide excretion were determined by absorption in weighable containers, correcting for the changes in composition of the air in the closed system from the beginning to the end of the period. Oxygen was admitted from a weighed cylinder and the oxygen absorption determined from the changes in composition of the air in the system and the oxygen admitted from the weighed cylinder. In 1913 (26) observations were begun at Bellevue Hospital, New York, with a closed-circuit respiration calorimeter for bedridden patients constructed by the Russell Sage Institute of Pathology on the same general principle as the respiration calorimeters in Boston.

In 1909 appeared the first description (27) from the Nutrition Laboratory of a respiration apparatus for the determination of the respiratory exchange and respiratory quotient of man in short periods. This apparatus was the outcome of the application of the principle used in the large respiration calorimeters in Wesleyan University at Middletown and in the Nutrition Laboratory at Boston. The general principle was the determination of the carbon-dioxide elimination by absorbing it in soda-lime in weighable containers. Oxygen was admitted to the apparatus from a weighed cylinder to compensate for the amount absorbed by the subject, and the indicator for the admission of oxygen was the rise and fall of a bathing cap connected with the circulating air. The attachment to the subject was made by means of pneumatic nosepieces. This respiration apparatus was subsequently improved (28) and the bathing cap was replaced by a spirometer, the movements of which were recorded on a kymograph, thus giving an index of the character of the respiration. The volume of oxygen admitted was determined not by weighing the cylinder containing the supply, but by passing the oxygen through a carefully calibrated meter. This apparatus was called the "universal respiration apparatus" because the same arrangement

could be used for short periods with man, or could be attached to a chamber for obtaining the respiratory exchange of an animal.

In 1916 Benedict and Tompkins (29) described a clinical respiration chamber principally designed for the accurate determination of the respiratory quotient in periods of one hour or longer. This utilized a form of the universal respiration apparatus, but the chamber was of the smallest capacity that could be used and still have a subject comfortable inside it.

In 1918 Benedict (30) reduced the universal apparatus to a much smaller form. Calcium chloride was used instead of sulphuric acid for absorption of water vapor, and the apparatus was so compact that it was transportable, that is to say, it could be moved from one room to another in a hospital without dismantling and without extra assistance. This still permitted the determination of the respiratory quotient as well as the oxygen absorption.

In 1920 (31) the portable or transportable apparatus was somewhat more simplified by eliminating the determination of the carbon-dioxide excretion and making the apparatus suitable for the determination of oxygen only. This apparatus was strictly portable, as it could be shortened or altered in such a way that transportation could be made by hand.

A simple and inexpensive apparatus for use by students was described by F. G. and C. G. Benedict (32) in 1923. The inauguration of the studies on racial metabolism led to the development of a field form of the student apparatus that could be easily disassembled, transported, and reassembled in the field for use in measurements of the metabolism of different races (33). This has proved of immense practical value and has functioned satisfactorily in studies of the basal metabolism of different racial groups all over the world and under unusual conditions.

The closed-circuit apparatus for the determination of the respiratory exchange of man in short periods has found wide application in European laboratories as well as American institutions. During the last decade a number of modifications have been devised, with especial reference to the determination of both carbon dioxide and oxygen. Among these may be mentioned those of Hagedorn (34), of Knipping (35), and of Helmreich and Wagner (36) in 1924, and those of Dethloff (37), and of Dusser de Barenne and Burger (38) in 1925. However,

we have come to the conclusion that the best method of determining the respiratory quotient, when this is necessary, is by the use of the helmet of Benedict (39) with an open-circuit apparatus, two blowers, and a spirometer or bathing cap, and meters. An aliquot sample is analyzed by the gas-analysis apparatus devised in the Nutrition Laboratory (40). With this apparatus it is possible to determine the carbon-dioxide and oxygen content of air to 0.003 per cent. Hence the apparatus can be ventilated at a rate rapid enough so that the carbon-dioxide content of the outcoming air will be under one per cent. This system gives the optimum opportunity for normal breathing and repiratory quotients with a high degree of accuracy.

Each one of us is losing weight at the present moment. On the average this loss amounts to about one-half gram per minute. This loss in weight, which is called the "insensible perspiration," is due to the fact that the sum of the vaporization of water and the elimination of carbon dioxide is greater than the absorption of oxygen. F. G. Benedict (41) has been investigating this loss in weight for a number of years. By means of a delicate balance he was able to measure the loss of a subject in periods as short as 10 minutes. In a cooperative research with Dr. H. F. Root (42) at the New England Deaconess Hospital in Boston, a relationship has been established between the hourly insensible loss and the metabolism. This method of estimating the basal metabolism promises to be useful with patients who can not or will not tolerate a direct measurement of the respiratory exchange.

Thus far we have dealt only with apparatus for determination of the respiratory exchange. At the beginning of this lecture I pointed out the connection between respiratory exchange and heat production first conceived by Lavoisier. Relatively few apparatus have been constructed for the direct determination of heat elimination and heat production in man. In 1892 Atwater and Rosa (43) began the construction of a respiration calorimeter at Wesleyan University, Middletown, Connecticut. Subsequently this was brought to a high state of perfection by Atwater and Benedict (44). In 1907 and 1908 calorimeters on the same principle were built in Boston at the Nutrition Laboratory (45). One was a bed calorimeter for subjects in the reclining position, and with this many of the earlier observations on the basal metabolism of humans were

obtained. Subsequently, as mentioned before, a respiration calorimeter was constructed by Du Bois and his associates at the Russell Sage Institute of Pathology in New York, and this has been much used in studies in disease.

In 1924 Noyons (46) described a calorimeter constructed on the differential or comparison principle. For a number of years at the Nutrition Laboratory there has been in use a calorimeter for humans on the differential or comparison principle. Although the observations with calorimeters have not been so numerous as those with the respiration apparatus, they have been of fundamental importance in establishing the general principle that the heat production obtained by the indirect method, that is, from the respiratory exchange, is comparable with that obtained directly by the calorimeters.

Choice of apparatus. In the determination of the basal metabolism, one of the first problems encountered is the choice of apparatus. Should one buy a simple portable type of apparatus for the measurement of oxygen absorption only, or should one secure more complicated apparatus necessitating a gas-analysis apparatus and gasometers for the open-circuit method and for the determination of the respiratory quotient? Only a few institutions determine the basal metabolism by the direct measurement of heat production, because of the large amount of funds and the large personnel required for such observations. Before considering the question of the apparatus to secure, we should have in mind what we are trying to measure. In the body three classes of substances are burned, protein, fat, and carbohydrate. Each of these has a specific amount of heat production per unit of material, and in the process of generating heat, carbon dioxide is produced as the result of oxidation and oxygen is absorbed in order to carry on the process. Table I shows the volume of carbon dioxide given off and oxygen absorbed and the heat produced in the body in the combustion of 1 gram of each of these three substances. Now we may measure directly the heat elimination and with suitable correction for the change in body temperature we may obtain the heat production, but as already pointed out, this is an expensive and complicated procedure. At the present time, therefore, the heat production is usually determined indirectly, that is, by measurement of the respiratory exchange. Along with measurements of the carbon-dioxide production and the oxygen consumption, some investigators

also determine the nitrogen eliminated in the urine, since the latter comes specifically from the metabolism of protein. From these measurements the heat production is calculated as follows: The grams of nitrogen in the urine are multiplied by the factor 6.25 to obtain the grams of protein burned. The value for grams of protein burned is then multiplied by the theoretical volume of carbon dioxide produced and by the theoretical volume of oxygen absorbed in the combustion of one gram of protein. The volumes of carbon dioxide and oxygen thus obtained are substracted from the measured total volume of oxygen consumed and the measured total volume of carbon dioxide produced. The results represent the sums, respectively,

TABLE I.

GASEOUS EXCHANGE AND HEAT PRODUCTION OF CARBOHYDRATE, FAT, AND PROTEIN.

(Amounts per gram.)

NUTRIENT	CO_2 cc.	O_2 cc.	R. Q.	CALORIES
Human fat	1,420	1,990	0.713	9.54
Glycogen	829	829	1.000	4.20
Protein	774	957	0.809	4.40

of the carbon dioxide production and the oxygen absorption due to the combustion of carbohydrate and fat. By means of simultaneous equations we may then calculate the weights of fat and carbohydrate burned. Finally, from the known caloric values of protein, fat, and carbohydrate we can determine the heat due to the combustion of each of the three substances. The sum of these heat values gives the total amount of heat produced as obtained by the indirect method.

This method necessitates time-consuming and tedious calculations in addition to the measurement of the respiratory exchange and the determination of the nitrogen in urine. If all basal metabolism measurements required this complicated procedure the number of metabolism measurements that could be made would be reduced considerably. An alternative method is to measure both the oxygen consumption and the carbon-dioxide production but to disregard the protein metabolism because it usually constitutes only 15 per cent of the total metabolism and because in the combustion of protein the caloric

value of oxygen per liter is nearly like that in the combustion of fat and of carbohydrate and the respiratory quotient is between that of fat and that of carbohydrate. A third and still simpler method is to disregard the determination of the respiratory quotient and measure only the oxygen absorption. The question arises as to what justification there is for either neglecting the protein or for assuming that one can obtain reliable and accurate results from the measurement of oxygen absorption alone. A number of writers on the subject of metabolism have pointed out that there is a wide variation in the possible ranges of respiratory quotients and have implied that there is the same wide range, percentagewise, in the heat production under the different respiratory quotients. However, one can see from the values in the table that between the quotients of 0.70 and 1.00 the range in caloric value of oxygen is not over 7 per cent. Hence it is the usual practice to calculate the heat production from the measured oxygen consumption, using the caloric value of oxygen at an assumed respiratory quotient of 0.82, namely, 4.825 calories per liter.

The question is, how close do we obtain the true heat production from the determination of the oxygen consumption alone? To answer this question, we have calculated, from an example from our own work, the heat production according to the three different methods of measurement just described.

EXAMPLE OF THREE DIFFERENT METHODS OF CALCULATING TOTAL HEAT PRODUCTION.

(Values per minute.)

156 c. c. CO_2	Nitrogen in urine
185 c. c. O_2	0.00625 gram
0.84 R. Q.	

Heat production calculated from

Protein, fat, and carbohydrate	= 0.905 cal.
Measured O_2 and measured R. Q.	= .898 cal.
Measured O_2 and assumed R. Q. of 0.82	= .893 cal.

In the example we had at our disposal for calculation the following factors: 156 c.c. of carbon dioxide per minute, 185 c.c. of oxygen per minute, with a consequent respiratory quotient of 0.84 and a nitrogen elimination of 0.00625 gram per minute. If the protein metabolism is taken into account, the heat production calculated from these figures is 0.905 calorie per minute. If we accept the idea that the respiratory quotient is necessary for the exact calculation of the heat production and use the

caloric value of oxygen at the respiratory quotient obtained in this case, we shall have 0.898 calorie. In this result we have employed the same values for carbon dioxide and for oxygen as we did in the first condition. In the third method of calculation we have used the oxygen measurement alone and have assumed a respiratory quotient of 0.82. Multiplying the oxygen figure by its caloric value for this respiratory quotient we obtain 0.893 calorie. Therefore, the three different methods of deriving the heat production give results ranging from 0.893 to 0.905 calorie per minute. One is justified in using methods that involve only the determination of oxygen for obtaining the basal heat production.

Having come to the conclusion that we are to measure the basal heat production by the indirect method and by the determination of the oxygen consumption alone, we still have further the question of selection of apparatus. For the average clinic the Roth modification (47) of the Benedict apparatus would appear to be the simplest form. This, however, requires effort on the part of the subject to open valves and to raise and lower a spirometer. For the most normal breathing conditions, we believe that an apparatus with a motor device is more suitable. Indeed, the best for this purpose is the apparatus modified in recent years by Benedict (48), in which a soda-lime container and a rotary blower are used and a spirometer serves as a dead end to the system. If a helmet is used as a breathing appliance instead of the mouthpiece, there is the greatest freedom from annoying conditions such as flow of saliva, uncomfortableness of the mouthpiece and particularly the noseclip. In recent series of observations, the helmet has been shown to be reliable and extremely comfortable, in fact, practically all individuals who have tried it prefer it to the mouthpiece. All apparatus of the spirometer type should have in connection with them a kymograph record of the excursions of the spirometer bell. If funds are limited, or if the work is in the field, and great portability is desired, the Benedict field respiration apparatus (33) is undoubtedly extremely useful. Indeed, this apparatus has proved itself of value already in a number of investigations in the field, notably in the West Indies and in Yucatan.

Normal test of apparatus. Every laboratory working on the determination of basal metabolism should test the accuracy of its apparatus with a normal subject. When the fact is once

established that the apparatus will give results on this normal subject within what is considered the normal range, this same subject's metabolism should be measured periodically in order to control the continued correct functioning of the apparatus. It is not sufficient to test the apparatus solely at the beginning of a series of measurements or when it is first installed. This subject should be called upon to act as a control whenever there is doubt as to the measurements which have been made upon a patient, and when such a person is used as a control the test should be made under as nearly as possible the same conditions as those which prevailed when apparently abnormal results were obtained with a patient. As Du Bois has pointed out, no work is better than its controls.

Theoretical test of apparatus. The best theoretical test of any type of respiration apparatus is the burning of a definite quantity of ethyl alcohol. The most all-round useful apparatus for this purpose is the combination of kymograph (49) or windshield wiper (50) and burette and spirometer, in which the small spirometer acts as a breathing apparatus and the burette is raised gradually so that the amount of alcohol burned can be measured.

Testing of apparatus during experiment. Much of the earlier work has been vitiated by the lack of an adequate test for the tightness of the apparatus during the actual measurement. With the closed-circuit and spirometer unit this test can be easily made by placing 30 grams (51) on the spirometer bell and observing whether there is any change in the level of the bell, either as indicated by the kymograph curve or by readings on the millimeter scale of the spirometer. If the slope of the kymograph curve or the curve of the plotted readings during that portion of the period when the weight is on the bell is different from the slope of the curve when the weight is not on the bell, this is proof of the presence of a continuous leak. The occurrence of an occasional leak (such as might be caused by the opening of the mouth or, in the case of the helmet, by movement of the head so that a slight opening is temporarily made in the closure around the neck) will be shown only on the kymograph record and will be indicated by a change in the trend of the curve.

Calculation of results. For the calculation of results a large number of charts, tables, nomograms, and other aids are now available. These are useful for saving time, but anyone who

is using a metabolism apparatus should be familiar with and capable of carrying out the calculations without the assistance of the charts and nomograms, even to the extent of doing all the calculations by arithmetic. This is particularly true with regard to technicians who have little knowledge frequently as to the significance of the figures that they are using, and who would in many cases never recognize ludicrous errors in either observations or calculations. It ought to be a regular routine in a laboratory for the operator periodically to carry out calculations by the long process, in order to have a thorough understanding of the methods of calculation and to acquire the ability to recognize abnormal figures when they are recorded.

CONCLUSION.

We have thus traced, in outline at least, the development of the modern respiration apparatus for the determination of basal metabolism of man. We have seen that with extremely simple and relatively inexpensive apparatus, the fundamental observations of the basal metabolism of man can be made in a relatively short period of time. However, we must not be misled by the idea that because the procedure is simple, a lack of rigid, careful attention to details of technique can be tolerated. We should regard the determination of basal metabolism with just as much seriousness of purpose as we would the more complicated and more laborious researches on the balance of income and outgo of matter and energy, from which today's simplification of technique is a logical descendant.

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RESPIRATION APPARATUS

Fig. 1. Apparatus of Lavoisier and Seguin (1789).
Fig. 2. Apparatus of E. Smith (1859).
Fig. 3. Apparatus of P. Regnard (1879).
Fig. 4. Apparatus of C. Speck (1892).

Fig. 5. Closed-circuit respiration apparatus (Benedict, 1909).

Fig. 6. Field respiration apparatus (Benedict, 1927).

Fig. 7. Helmet with closed-circuit respiration apparatus (Benedict, 1930).

Fig. 8. Helmet with open-circuit respiration apparatus (Benedict, 1933).

PROBLEMS IN THE DETERMINATION OF THE BASAL METABOLISM OF MAN AND FACTORS AFFECTING IT

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Our lives are continually maintained by the processes of heat production. As long as we produce heat, we are alive, and when heat production stops, living ceases. Thus the end result of our various activities is heat production. There is a minimum level of heat production below which we can not live for any length of time. This minimum level of heat production is the result of internal glandular and cellular activity, and of respiration and circulation. When we measure this heat production with the body in a resting, quiet condition without external muscular activity, and after the effects of the last ingested food have ceased, we call it the basal metabolism. We shall consider in this lecture some of the problems in the measurement of basal metabolism and some of the factors that affect the basal metabolism.

Effect of neutral bath. Ordinarily the basal metabolism is measured with the subject in a comfortable condition so far as the sensation of cold or warmth is concerned. In the laboratory this usually means with the ordinary clothing and, if the temperature is about 20° Centigrade, there may be a light covering of blankets. Usually, however, the amount of clothing and covering of the subject during basal metabolism measurements is not reported by the investigator, and Lefèvre (1) has criticized this procedure. He maintained that in practically all basal metabolism measurements, as ordinarily made, the conditions for heat loss are such that the metabolism is higher than the true basal. According to him the true basal metabolism can be obtained only when the subject is immersed in a water bath at 35° to 36° C., because under other conditions there will be an excess heat production to combat the loss of heat to the environment. To meet this criticism tests were made by F. G. and C. G. Benedict (2) with several subjects in which the basal metabolism was first determined

with the subjects lying clothed and lightly covered at a temperature of 15°, in order to accentuate the possible influence of a cool environmental temperature. After these measurements were made, the subjects entered a neutral bath at 34° to 35° C. in a warm room and the observations were repeated under these conditions.

The results in Table I indicate that there was no decrease in the metabolism due to immersion in a bath at nearly body temperature. If anything, there was a slight tendency to an increase in the metabolism. After the bath the subject's metabolism returned to the basal value quickly. The contention of Lefèvre was thus not substantiated. It is obvious that the determination of basal metabolism by immersion in a

TABLE I.

Mr. B; Age, 47 Years; Nude Weight, 67 kg.; Height, 169 cms.

(Oxygen consumption in c. c. per minute.)

Date	Basal (Room Temp. 16°–18° C.)			Bath at circa 36.0° C.					
October 4	204	205	197	208	215	210	202	.....	.....
October 5	203	207	200	211	222	223	224	230	.....
October 6	196	220	213	212	213	215	211	236	224

bath would be highly impracticable. Furthermore, the tendency for the metabolism to increase during the bath emphasizes the necessity for not having too heavy covering on the subject, as this might lead to a physiological fever with a consequent rise in metabolism.

Minor muscular movements. It is generally recommended that the subject remain perfectly quiet during the period of measurement. It is difficult for some people to understand what perfectly quiet means. They are apt to change the position of the legs, or to raise the hand to the face to scratch, if a slight itching or irritation takes place. One always seems to have a desire to do these things just at the wrong time. Lefèvre of Paris criticized the emphasis laid on the complete absence of muscular activity and calculated from the foot pounds required to raise the hand to the head that it would require an extremely small amount of energy. In order to test the effect of slight movements on the metabolism, F. G.

and C. G. Benedict (3) studied two well-trained subjects in the Nutrition Laboratory in which measurements were made under basal conditions and then during periods when the subject raised the hand to the forehead every four seconds. The results are shown in Table II.

TABLE II.

INFLUENCE UPON THE OXYGEN CONSUMPTION OF SMALL MUSCULAR MOVEMENTS OF ARMS AND LEGS.

SUBJECT A			SUBJECT B		
Date and Condition[1] 1924	Period	Oxygen Consumption per Minute c. c.	Date and Condition[1] 1924	Period	Oxygen Consumption per Minute c. c.
January 3			February 29		
Basal	I	193	Basal	I	256
Basal	II	188	Basal	II	255
Arm movement	III	218	Basal	III	253
Basal	IV	195	Arm movement	IV	280
January 7			March 1		
Basal	I	200	Basal	I	257
Basal	II	196	Basal	II	255
Arm movement	III	210	Basal	III	244
Basal	IV	193	Arm movement	IV	268
Leg movement	V	222	Leg movement	V	285
January 9					
Basal	I	203			
Basal	II	189			
Arm movement	III	224			
Leg movement	IV	209			
Basal	V	189			
January 16					
Basal	I	202			
Basal	II	195			
Arm movement	III	215			
Leg movement	IV	225			
Basal	V	200			

[1]In basal periods subjects were lying, clothed, and covered with light blanket. In the arm movements the hand was raised to the forehead every 4 seconds. In the leg movements the feet were crossed every 20 seconds.

The actual increase proved to be only 1.5 cc. of oxygen for each movement. In another series, the subject crossed and uncrossed the legs every 20 seconds. One such leg movement every minute would raise noticeably the basal metabolism. Although single minor muscular movements, such as raising the arm to the head, have but little influence on the basal

metabolism, movements of the legs must be denied. It is unsafe to disregard any of the stringent rules for quiet muscular repose. It is best to do everything possible to get the subject into a comfortable, relaxed position so that there will be no desire to change the position. The operator should have everything as free as possible from little annoyances, such as wrong lights, jarring the bed, and slamming doors.

Effect of previous activity. Benedict and Crofts (4) showed that the basal metabolism was not raised by the muscular exercise of rising, bathing, dressing, walking in wintry weather for 10 minutes, and climbing 3 flights of stairs, provided that

TABLE III.

OXYGEN ABSORPTION AND PULSE RATE PER MINUTE OF COLLEGE WOMEN BEFORE AND AFTER RISING.

SUBJECT No.	OXYGEN ABSORPTION		PULSE RATE	
	Before Rising	After Rising	Before Rising	After Rising
	c. c.	c. c.		
II	243	249	56	51
III	193	196	65	63
IV	190	200	56	55
V	173	175	57	56
VI	170	170	59	56
VII	182	186	59	57
VIII	170	165	62	58

after such exercise the subject was clothed and lay quietly for 30 minutes, lightly covered, in a room at circa 20° C. Values secured under such conditions were compared with those obtained after a night's sleep in bed before the subjects arose and went through the activity previously described. Table III gives a summary of the results.

Constancy from hour to hour. Some of the questions that arise in connection with the determination of basal metabolism are, how many periods of measurement shall there be on the same day, and how shall we make a selection from the results, or shall we average all that we obtain? Certainly we should not depend on one observation and it is better to have three periods, if possible. Then we may consider the first period as one of adjustment and average the results of the other two. Some years ago, in a study of the comparative accuracy of

different types of respiration apparatus, (5) I came to the conclusion that in three successive periods the oxygen consumption should not have a greater range than 10 c.c. This would mean a 5 per cent range, if the average oxygen consumption were 200 c.c. per minute. Since that time I have come to the conclusion that this is too rigid a standard to demand in all cases, and that with untrained subjects a greater range would have to be allowed, probably 15 c.c. at a level of 200 c.c. per minute. As a general rule, it is not wise to make observations for more than three periods, rejecting the first if materially higher than the others, or averaging all three. Subjects usually become fatigued or restless after three periods,

TABLE IV.

CONSTANCY IN BASAL METABOLISM IN CONSECUTIVE 10-MINUTE PERIODS.

(Mr. C., January 26, 1925.)

PERIOD	OXYGEN CONSUMED PER MINUTE	PERIOD	OXYGEN CONSUMED PER MINUTE
	c. c.		c. c.
I	219	VII	225
II	220	VIII	229
III	219	IX	223
IV	230	X	223
V	223	XI	222
VI	225		

and then the metabolism rises. Table IV shows that with a well-trained subject it is possible to obtain a series of 10 periods with a narrow range in values (6).

Constancy from day to day. Not only are we concerned in the constancy of the basal metabolism on the day of measurement, but also the range of values from day to day is of importance in any study of metabolism. It is the practice in some laboratories to determine the basal metabolism on several days and then use the average of these days' measurements as a base-line for comparison with measurements under other conditions on days when the determination of the basal metabolism is omitted. This may be justifiable when the effect of the superimposed factor is large, but hardly acceptable when only slight differences are expected. As a rule, rarely, if ever, do we use an average basal value derived from measurements on several days to calculate the effect of a superimposed factor.

It is always best to determine the basal metabolism on the same day and under the same conditions as those under which the superimposed factor is to be studied. In 1921 a statistical analysis made by Harris and Benedict (7) on 11 subjects who had been studied from 20 to 53 days led to the general finding

TABLE V.

Variations in Oxygen Absorption from Day to Day.

Subject	Number of Experiments	Range of O_2	Average O_2	Coefficient of Variation
		c. c.	c. c.	
J. C.	40	198–231	210	3.4
J. C.	55	202–235	215	3.5
J. C.	31	207–232	222	2.4
J. C.	36	210–251	227	3.4
C. M. B.	41	238–283	257	3.6

of a coefficient of variation of 4 per cent of the average metabolism. During the past few years we have had several series of experiments with one subject and one series with another varying from 31 to 55 days. Table V shows the results, which are of about the same order as those obtained by Harris and

TABLE VI.

Constancy in Basal Metabolism on Consecutive Days.

(Mr. C.)

Date	Oxygen Consumed Per Minute	Date	Oxygen Consumed Per Minute
	c. c.		c. c.
April 21	217	May 5	216
April 22	215	May 10	225
April 23	214	May 19	221
April 24	218	June 24	226
April 25	229	June 25	219
April 28	222		

Benedict. The highest result with C. M. B. was on the first day. In all of the measurements this subject was sitting but post-absorptive, as was also J. C. in the third series. An example of low daily variation is shown in Table VI, (8) when J. C. was studied on 11 different days.

Seasonal variations. In the course of the year there are marked variations in the external temperature, which in our latitude ranges from 100° F. in the shade with high humidity in summer to 10° below 0° F. with low humidity in winter. We try to accomodate ourselves to these conditions by change in amount and character of clothing, to some extent by lessening activity in summer, and also by an attempt at artificial cooling (consumption of iced drinks and foods). The question, however, arises as to whether there may not be a seasonal variation in basal metabolism which may occur in spite of our attempts to adapt ourselves to climatic and seasonal changes. In order to obtain some information on the possible occurrence of a seasonal variation, Gustafson and Benedict (9) made a

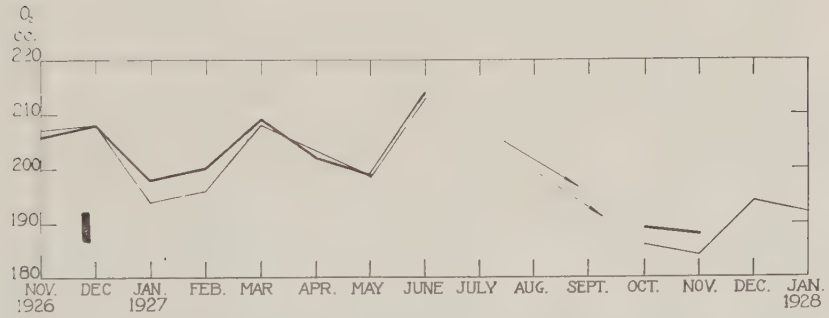

FIGURE 1. Course of the oxygen consumption of young women with the change in season. The light black curve represents the average for five subjects, the heavy black curve that for eight subjects.

series of measurements of the basal metabolism of 20 Wellesley College students once each month from October, 1926 to January, 1928 with the exception of July, August, and September. In Figure 1 the light line curve shows the course of the average basal oxygen absorption of five young women and the heavy line curve that of eight subjects. There was a low metabolism during the winter followed by a higher level in the spring. The low level in May may possibly be due to the relatively large number of observations during menstruation. In the course of a series of measurements on a well-trained male subject we have observed that the metabolism tends to be higher in the spring than at any other time. The cause for the change in metabolism is not known. It can scarcely be due to a rise in external temperature, as the temperature is higher later in the year.

Menstruation. When the basal metabolism of normal women between the ages of puberty and the menopause is determined, it is usually recommended that the observations be taken on days outside the menstrual period. A number of studies have been made on the basal metabolism during the menstrual period as compared with that during the non-menstrual interval. Hitchcock and Wardwell (10) in this institution have reported a series of 800 tests on 27 women. Selecting the results on 20 women, they found that 14 showed a lowering of the basal metabolism during the menstrual period. Benedict and Finn (11) studied the day to day varia-

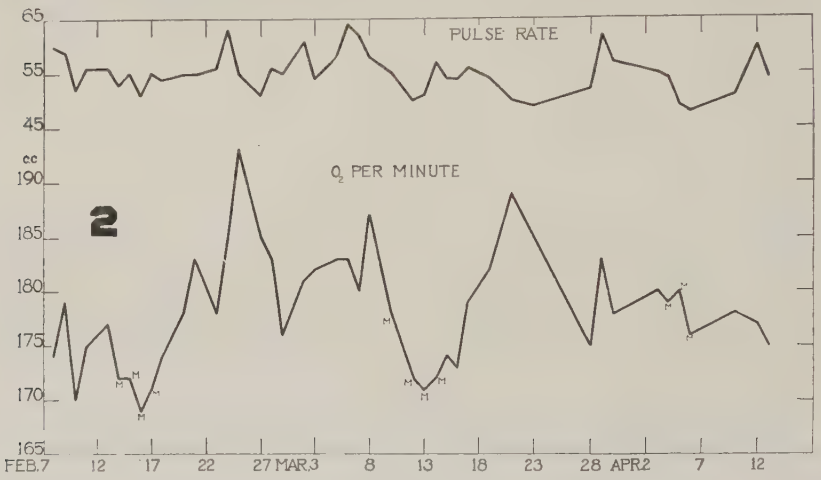

FIGURE 2. Oxygen consumption (cc.) and pulse rate per minute of Miss W. from day to day throughout a period of two months, including three menstrual cycles. The menstrual days are indicated on the oxygen curve by the letter M.

tion on a well-trained subject for two months, including three menstrual cycles. Figure 2 shows the daily variations in oxygen absorption and pulse rate for this subject covering more than two months. The menstrual days are designated M. In all three menstrual periods the metabolism was lower than that obtained during the remaining portion of the month.

Metabolism before and after vacation. In the course of a number of years, observations have been made on the metabolism of members of the staff of the Nutrition Laboratory (12) before vacation, usually in July, and then again as soon as possible on their return to laboratory activities, usually in September. The number of subjects studied varied from year

to year, and a series of observations was made in 4 different years, 1918, 1920, 1925, and 1927. In Table VII is given a summary of the average results obtained each year. The groups were not the same each year, and consequently the average values vary. The table includes data on the body weights and the pulse rates as well as the oxygen absorption. In general, most of the individuals gained weight during the vacation although this was not invariably the case. In some cases there were gains of as high as 4 kilos during vacation. This is reflected in the general average of the body weights. In spite of this, however, the averages for three of the four groups indicate practically no change in the oxygen absorption measured in the basal condition.

TABLE VII.

AVERAGE BASAL METABOLISM BEFORE AND AFTER A VACATION.

YEAR	NUMBER OF SUBJECTS	BODY WEIGHT (kilos)		OXYGEN PER MINUTE (c. c.)		PULSE RATE PER MINUTE	
		Before	After	Before	After	Before	After
1918.....	9	54.1	55.4	188	188	64	64
1920.....	5	56.5	58.3	195	189	65	64
1925.....	5	63.6	63.4	200	221	61	66
1927.....	9	60.8	61.3	187	190	63	62

In 1925 three of the group showed marked increases in metabolism. Thus, in one case the oxygen absorption increased from 142 to 172 cc. per minute and in another case from 254 to 273 cc., that is, gains of 21 and 8 per cent, respectively. In none of the individuals in the other three series was there any pronounced change in the metabolism. Also, in this group in 1925 there was a marked increase in pulse rate. Most of this increase, however, is due to a change in the pulse rate of one individual from 55 before vacation to 82 after vacation. One of the same subjects who showed a marked increase in 1925 also showed an increase in 1927.

These studies, however, indicate, as a whole, that the vacation does not result in any change in metabolism. This is surprising and speaks for a marked fixity in basal metabolism which seems to be unaltered even when pronounced subjective

impressions of recuperation and betterment are expressed. In view of the present fad for sun tanning it may be advisable or desirable to have additional studies in which the possible effect of exposure to sunlight is specifically studied.

Body temperature. It is well known that the metabolism is raised during fever. Du Bois (13) found that for each degree Fahrenheit rise in body temperature the metabolism was increased 7.2 per cent. The possibility of the variation in metabolism within normal range being due to variations in body temperature has been but little considered. It is our custom to take the temperature by mouth each day before any measurement of metabolism is made. This aids us in ruling out any measurement with febrile temperature. It is

TABLE VIII.

Correlation Between Mouth Temperature and Oxygen Absorption.

Subject	Number of Experiments	Range of O_2	Range of Temperature	Correlation Coefficient
		c. c.		r. P. E.$_r$.
J. C.	40	198–231	35.8–37.1° C.	+0.502 ± .080
J. C.	55	202–235	35.7–37.1° C.	+0.386 ± .077
J. C.	31	207–232	36.0–36.8° C.	+0.530 ± .087
C. M. B. ...	41	238–283	96.1–97.9° F.	+0.407 ± .088

recognized that temperatures by mouth are not so reliable as rectal temperatures, but at least an unusually high temperature is significant even when taken in this manner. In a study of the effect of sugars on metabolism and in a study of the effect of muscular work on sugars and on the metabolism of alcohol we have completed several series of experiments in which we have had both mouth temperatures at 8:30–9:00 a.m. and basal metabolism determinations shortly after. Table VIII shows the number of observations, range of oxygen absorption and of mouth temperatures, and the correlation coefficients between mouth temperature and oxygen absorption. Although only two of the coefficients are greater than 6 times the probable error, they are all positive and in the same direction, and indicate that the metabolism varies with mouth temperatures and that the latter are of some significance as an explanation of the variations in metabolism. The temperatures

at the upper part of the ranges are probably of more significance than those at the lower end. An early morning temperature of 96.1° F. undoubtedly is erroneous, whereas a temperature of 98.2° F. is of significance. It should be noted that but rarely is a "normal" body temperature of 98.6° obtained. In fact, 98.6° at 8:30 a.m. would be regarded by us as an indication of a mild febrile condition.

Effect of sleep. One of the conditions of the measurement of basal metabolism is that the subject shall not be asleep. It would be ideal if the measurement of the basal metabolism could be made with the subject asleep, because in this condition a person would be more likely to be completely relaxed and free from possible fear. The true difference, however, in different people between the metabolism asleep and that while awake is not so definitely known that we can apply a general value for making the comparison. The measurement of the oxygen absorption during sleep is difficult to make because the character of the respiration is likely to be irregular. Sleep affects the pulse rate and the respiratory quotient as well as the oxygen absorption. In general, the decrease in the metabolism due to sleep is about 10 per cent. Until recently measurement of the metabolism during sleep has been difficult, because of the lack of a suitable breathing appliance. None of the breathing appliances used for short periods has been reliable enough to prevent leakage during sleep. The helmet recently devised by F. G. Benedict (14) now makes it possible to study the metabolism during periods of sleep and periods when the subject is wide awake.

Hypnotic sleep. Whitehorn, Lundholm, Fox, and Benedict (15) found that simple hypnotic sleep might have an influence on basal metabolism by reducing the high figures obtained in training subjects, but did not reduce the rate below the normal value. In this respect, it differs from normal sleep.

Effect of mental effort. Basal metabolism is measured when the subject is awake, and under this condition, the mind is more or less active. It may wander from one thing to another or the person may concentrate intensely on a problem he has on hand in order to disassociate himself from the measurement and to prevent himself from becoming bored. A question that is frequently asked is, does mental "work" increase metabolism? Recently F. G. and C. G. Benedict (16) conducted a series of studies on six subjects to determine the effect

of intense mental effort on the basal metabolism. The metabolism was first measured with the subject in a comfortable, reclining position, and in so far as possible, during mental vacuity. Then for one hour the subject was given mental arithmetic by a person reading problems of multiplication, such as 76 × 69. The subject signalled when the problem was solved, and was given another immediately. The metabolism was measured with the helmet respiration apparatus, both on the closed-circuit principle and on the open-circuit principle, with gas analysis. Table IX shows the effect of this intense sustained mental effort on the metabolism. On

TABLE IX.

EFFECT OF MENTAL EFFORT ON OXYGEN CONSUMED.

(c. c. per minute.)

SUBJECT	REST	WORK
I	208	210
II	212	219
III	232	241
IV	242	247
V	174	187
VI	181	191
Average	208	216

the average, the oxygen consumption was increased less than 4 per cent.

Standards of basal metabolism. For a number of years the Nutrition Laboratory collected data on the basal metabolism of normal men and women. In 1919 Harris and Benedict (17) evolved a set of prediction formulas for the average basal metabolism of men and women. These took into account the effect of height, weight and age and difference in sex. These formulas were based on metabolism data on 136 men and 103 women. Two other standards are also available, the Aub and Du Bois (18) based on body surface, and the Dreyer (19) based on age and weight. In general, the three standards give about the same results, because they are based upon practically the same original material. Benedict (20) has recently reported additional measurements on 34 men and 32 women. In both groups the average variation of the measured metabolism from the predicted was less by the Harris and

Benedict and Dreyer than by the Aub and Du Bois standards. It is found that the present standards for women are about 5 per cent too high. Benedict reiterates his belief that the heat production is determined by the active mass of protoplasmic tissue and by some existing stimulus to cellular activity. He is strongly inclined "to support the belief that differences in basal metabolism are more logically interpreted with reference to differences in age, height, weight, and sex rather than with reference to differences in surface area."

Effect of age on basal metabolism. Although the basal prediction formulas include a factor for the effect of age, this

TABLE X.

AGE AND BASAL METABOLISM. CALORIES PER KILOGRAM PER DAY.

Miss W.		H. M. S.	
Age	Calories	Age	Calories
24	23.0	43	22.8
27	22.5	48	22.8
29	22.0	50	23.0
30	22.2	59	22.0
31	22.2	64	19.7
32	22.5		
33	22.8		
34	20.9		
35	21.0		
36	20.8		
37	19.9		
41	20.3		

factor is applicable to groups as a whole. So little is known about the effect of age with given individuals that no prediction can be made at the present time as to the possible variations in the effect of age on individuals who, although increasing in years, may still be in practically good health.

The Nutrition Laboratory has had the opportunity to follow the metabolism of 4 individuals for periods of from 17 to 24 years (21). Table X shows the series of values for the heat production per kilogram per day of a woman measured over a period of 17 years and for a man measured over 21 years. On the woman the series began in 1916 and on the man, in 1911. In the case of the woman there is a slight

decrease in the metabolism per kilogram. Part of this decrease probably can be ascribed to a slight increase in weight, as she changed from a value in 1918 of 54 kilograms to a value of 60.5 kilograms in 1928.

With the man there has also been a gain, from 59 to 63.6 kilograms from 1911 to 1932, and there has been but little change in total calories per kilogram for the entire period of time.

TABLE XI.

AGE AND BASAL METABOLISM. CALORIES PER KILOGRAM PER DAY.

T. M. C.		F. G. B.	
Age	Calories	Age	Calories
30	27.8	38	22.5
32	26.4	39	21.6
34	27.8	40	21.6
35	26.6	41	20.6
36	26.9	44	20.7
40	26.5	45	21.1
42	24.6	46	20.8
46	22.8	47	21.0
49	22.9	48	20.6
54	23.5	50	21.1
		51	21.3
		52	23.3
		53	21.9
		55	20.4
		57	19.8
		61	20.1
		62	20.6

Table XI gives the results obtained on two members of the staff who have been connected with the Nutrition Laboratory since its beginning in 1907. With T. M. C. the first observations were in 1909, and there is a marked decrease in the values per kilogram from 27.8 to 23.5 calories at the age of 54. During this period of time the maximum change in weight was 3.5 kilograms, and there was an actual difference between the beginning weight and the last weight of 0.7 kilogram. The most marked decrease in metabolism was at the age of 46. The other subject shows likewise a decrease in metabolism per kilogram, but not so marked as with T. M. C. In 1917 at the age of 39, T. M. C. had typhoid fever. Whether this

had any effect on the subsequent change in total metabolism cannot be stated, although for 6 months or more there was a marked decrease in activity. At the age of 44 T. M. C. purchased his first automobile and since that time has traveled almost exclusively by automobile rather than on foot and in street cars, as formerly. It is not improbable that some of the decrease in metabolism may have been due to a decrease in activity.

F. G. B. has been an extraordinarily active man during the entire period of observation. In fact, it is due to his inexhaustible energy and capacity for work and his continuous ingenuity in devising apparatus for over 25 years that most of the material is available for the substance of these two lectures.

The actual decrease in total calories for the two men over a period of 19 years was 283 calories for T. M. C. and 310 calories for F. G. B. In the Harris-Benedict prediction formula the same decrease per year is presented, regardless of the size of the individual. Many more observations are needed to establish the variations in the effect of age on the metabolism of the individual.

Elderly women. One of the marked gaps in the available data on the basal metabolism of normal humans is the lack of measurements beyond the age of 40. Recently Benedict and Meyer (22) determined the basal metabolism of 23 elderly women from 66 to 86 years of age and weighing from 32 to 72 kilograms. These women were all presumably in good health and none of them were bedridden. Most of them took care of their own rooms and some helped in the general care of the institution where they were living. On all bases of comparison the metabolism decreases with age. Beyond 78 years one might accept as a round figure 1000 *total* calories per 24 hours. Table XII shows the results of the measurements and the comparison by the three different standards of prediction. The variations from the predicted values for the different individuals were rather wide, from +14.9 to −16.9 per cent on the Harris-Benedict basis of prediction and the average deviation for the whole group was −0.8 per cent. The predicition by the Harris-Benedict standard was closer on the average than either of the other two bases of prediction. In general, there are such wide divergences that no existing standards may be considered to predict accurately the basal

metabolism of individual elderly women. Therefore, until many more have been studied and more exactly measured the prediction of metabolism in old age may not be accepted.

Metabolism of Orientals. The standards for the prediction of basal metabolism were based entirely on observations with Caucasians, and at the time they were formulated there was

TABLE XII.

Basal Metabolism of Elderly Women.

Subject	Age	Weight	Total Heat Produced per 24 Hrs.	Per Cent Deviation		
				Dreyer	Aub-DuBois	Harris-Benedict
	yrs.	kg.	cal.			
I	66	50	931	—18.3	—24.0	—16.9
II	68	71	1359	+ 1.0	— 2.0	+ 4.6
III	70	72	1401	+ 3.6	+ 2.2	+ 7.1
IV	70	52	1112	— 3.6	— 3.9	+ 0.5
V	71	72	1501	+11.2	+ 8.3	+14.9
VI	71	64	1149	— 9.5	— 9.9	— 5.5
VII	71	67	1323	+ 2.0	— 5.1	+ 4.1
VIII	71	53	1096	— 5.3	— 8.4	— 2.1
IX	73	64	1318	+ 4.1	+ 0.9	+ 8.4
X	74	63	1122	—10.6	— 9.8	— 5.5
XI	74	45	936	—11.5	—17.4	— 9.2
XII	76	49	1148	+ 3.7	+ 1.4	+ 8.9
XIII	77	32	799	— 9.9	— 9.9	— 6.4
XIV	78	42	973	— 3.9	— 5.5	+ 1.8
XV	79	69	1049	—19.5	—19.7	—14.5
XVI	81	67	1035	—19.0	—15.7	—12.8
XVII	81	41	961	— 3.4	— 5.9	+ 1.6
XVIII	84	50	1015	— 7.9	— 6.3	+ 0.3
XIX	84	44	1045	+ 1.4	— 3.5	+ 7.2
XX	84	44	1070	+ 3.2	+ 5.6	+12.2
XXI	84	45	973	— 7.1	— 8.2	— 0.3
XXII	84	63	966	—21.9	—19.4	—15.3
XXIII	86	54	1026	— 9.7	— 9.7	— 2.0
Grand Average (Per Cent)				— 5.7	— 7.2	— 0.8

no indication but that they were applicable to all other races. However, in 1924 and 1925 MacLeod, Crofts, and Benedict (23) made some observations on the basal metabolism of 7 Chinese and 2 Japanese women, ages 21 to 29 years, who resided either at Mount Holyoke or Columbia University. It was found that the basal metabolism of these women was from 2.3 to 16.5 per cent below the Harris and Benedict standards, on the average 10.4 per cent below. Since these

young women had been living under the same conditions as the other students at the institutions and having the same diet, it was suggested at that time that the metabolism of Orientals may be specifically lower than the English and American standards and that a racial effect in the direction of a lower metabolism be recognized with the Chinese and the Japanese.

Racial variation in metabolism. The observations of MacLeod, Crofts, and Benedict led the Carnegie Institution of Washington to undertake an extensive research in cooperation with investigators of other institutions on the basal metabolism

TABLE XIII.

MALE MAYAS IN YUCATAN. DEVIATION OF MEASURED METABOLISM FROM AMERICAN STANDARDS.

INVESTIGATOR	PER CENT DEVIATION
Williams	+5.2
Shattuck	+5.8
Steggerda:	
First Day +9.2	
Second Day +7.7	
Third Day +8.2	
Average	+8.4
GRAND AVERAGE	+6.5

of a number of different races. On the occasion of an expedition to Yucatan in 1927, sent out by the Carnegie Institution of Washington and Harvard University, Dr. George D. Williams measured the metabolism of 32 male Mayas and found that the metabolism of the Mayas was, on the average, 5.2 per cent above the standards for white men of similar ages, heights, and weights (24). Three years later a second expedition to Yucatan was organized by Harvard University, working under the auspices of the Carnegie Institution of Washington. On this expedition measurements of the metabolism were made on 25 male Mayas under the direction of Dr. George C. Shattuck (25). Many of the subjects were the same as those that had been studied by Williams. The grand average deviation was +5.8% for the 25 individuals.

In a third expedition to Yucatan in 1931, carried out by the Department of Genetics of the Carnegie Institution of Washington, Dr. Morris Steggerda (26) measured the metabolism of 30 men and in order to solve the question as to whether the previous values may have been due to measurements only on one day, the observations were repeated on three different days. The grand average of all the values for percentage deviation of measured from predicted metabolism was +8.4. Table XIII shows the averages obtained on the three different expeditions. Coincident with these high values of metabolism

TABLE XIV.

Female Tamils in Madras. Deviation of Measured Metabolism from American Standards.

Subject	Per Cent Deviation	Subject	Per Cent Deviation
1	—16.6	15	—17.3
2	—15.8	16	—26.5
3	—13.0	17	—15.0
4	—10.5	18	— 9.8
5	—18.1	19	—21.9
6	—19.2	20	—13.0
7	—21.4	21	—17.5
8	—16.7	22	—16.0
9	—12.3	23	—20.2
10	—20.5	24	—19.7
11	—12.1	25	—17.8
12	—18.0	26	—14.2
13	—25.2	27	—10.6
14	—29.8		

Grand Average, —17.4

were low pulse rates, in fact, lower than usually observed with whites. In the last series many of the pulse rates were 45 or below. Similar low values were obtained by Shattuck and Williams, indicating that the high metabolism was in no way due to high heart rate.

In contrast to the metabolism of the male Mayas is the low metabolism of female Tamils measured by Professor Eleanor D. Mason at the Women's Christian College at Madras (27). Twenty-seven female Tamils were found to have invariably a low metabolism compared with the prediction. Table XIV shows the individual deviations for the 27 subjects

and the average of the group as a whole, namely, −17.4 per cent. In the same institution another group of South Indian women, 17 Malayalis, showed an average of −16.1 per cent below the standards for American women.

These studies all indicate significant differences from American standards in the metabolism of the different races. The problem of variation in basal metabolism according to race is complicated by the factors of climate and of diet. However, the climate in South India and the climate in Yucatan are not so different as to suggest that climate plays the dominant rôle in the metabolism of these two races.

CONCLUSION.

We thus see that the determination of the basal metabolism is a fundamental measurement by which we may compare groups of individuals with respect to standards of nutrition, differences in race, effect of climate, and effects of such factors as influence human beings in masses. Knowledge of the basal metabolism is of such profound physiological importance that many more and careful measurements are needed before we can formulate the general laws governing the level of metabolism of each individual. Determination of the basal metabolism is of world-wide significance because it is applicable as a measure of vital activity to each human being.

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The Universe of Light.

The rapid advances in physical science have centered largely around the phenomenon of radiation. This book, written in popular style, presents by description, analogy and experiment a clear understanding of our everyday experience with light, color, and associated phenomena. To those who, while not physicists themselves, have followed the recent popular accounts of astronomy, this book will appeal as offering a wider range of discussion and analysis.—L. H. S.

The Universe of Light, by Sir William Bragg. x + 283 pp. New York, The Macmillan Co. 1933. $3.50.

Savagery to Civilization.

The "Century of Progress" is but a step on the long long trail over which the author takes us in review of anthropology. The trail leads through the hunting life of the Old Stone Age to the pastoral life of the early tillers of the soil; through the bronze age to the dawn of history. Villages become cities, Egypt, Babylon and Crete grow and wane, and modern man emerges to carry on and continue along the trail that leads into the future. The author is to be congratulated on his ability as a guide along the trail.—L. H. S.

The Long Road from Savagery to Civilization, by Fay-Cooper Cole. One of the Century of Progress Series. xi+100 pp. Baltimore, the Williams and Wilkins Co. 1933. $1.00.

Plant Genetics.

This book fills a long-felt need for a modern, technical discussion of the subject of plant genetics. The first three chapters deal comprehensively with "Inheritance in Diploids," "The Chromosome Theory of Heredity," and "Constitution of the Factor." The latter two of these chapters present a detailed summary of the cytological basis for hereditary behavior. Following are four chapters dealing with one aspect or another of the phenomenon of polyploidy. Fundamental as this concept is to plant genetics, it has been neglected in most books in the general field of genetics. There are also chapters on "Structural Hybrids" and "Interspecific Hybridisation." The book closes appropriately with a chapter dealing largely with the species problem. This discussion is very suggestive regarding the role which genetics may attain in placing taxonomy upon an experimental basis. There is a bibliography of over a thousand titles which should prove invaluable to research workers in the field of genetics; and an index. This book will fully justify the space it occupies on the reference shelf of any botanist or geneticist.—B. S. Meyer.

Recent Advances in Plant Genetics, by F. W. Sansome and J. Philp. x+414 pp. Philadelphia, P. Blakiston's, Son & Co. 1932.

SOME ASPECTS OF CARBOHYDRATE METABOLISM

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An eminent biochemist was wont to say facetiously that he had the ambition to make a lump of sugar and to lay an egg. He lived to fulfill the first of these aspirations, but died before he had the egg quite finished. I refer, of course, to Emil Fischer. He made not only one but several kinds of sugar and together with Kossel, about the beginning of this century, he revealed the fundamental nature of proteins. In carbohydrate metabolism one cannot omit the proteins, for carbohydrate can be formed from protein and this is an important source of biotic energy. Some reference will be made to this aspect of carbohydrate metabolism, but I shall be concerned mainly with the question of the possible formation of sugar from the other principal source of energy, namely, fat.

Metabolism properly begins with digestion. Let us look just a moment at the nature of this change and particularly its rate. The nature of the change is indicated by the reaction $(C_6H_{10}O_5)n + nH_2O \rightarrow (C_6H_{12}O_6)n$. This is called hydrolytic cleavage. All of the ordinary processes of digestion are of this nature.

Digestion of starch is begun by saliva in the mouth, provided the saliva is well mixed with the food. A familiar laboratory experiment to illustrate the rapidity of the action of ptyalin, the starch splitting ferment of the saliva, is to chew a soda biscuit or cracker for two minutes, place the chewed mass over a filter paper and wash with distilled water. Applying a copper reduction test to the filtrate one gets a good reaction for sugar.

Further evidence of the rapid action of saliva is obtained by digesting in glassware cooked cereal breakfast foods. The following Table (I) illustrates:

TABLE I.

DIGESTION FOR FIVE MINUTES AFTER COOKING FIFTEEN MINUTES.

	Complete Digestion.	Digestion to Solubility.
	%	%
Wheat endosperm	22.7	70.7
Precooked oats	34.9	77.2
"Whole wheat"	26.8	67.6
"Toasted whole wheat"	31.1	66.8

Biedermann speaks of the almost "explosive action" of ptyalin in digestion of starch. If the starch be already soluble the digestion to sugar is even more rapid.

Cannon at Harvard showed about 30 years ago that digestion of starch can proceed in the stomach after a mixed meal for from 15 to 90 minutes, being stopped only when the entire stomach contents become acid. This has been confirmed by Bergeim and Hawk, who used the retention tube and drew up samples of stomach contents at various intervals after digestion started. Under favorable circumstances therefore the starch may be digested to complete solubility therefore in the stomach. Disacchrides like cane sugar need only to be inverted, i. e., split into two molecules, for single sugars to be ready for absorption—

$$C_{12}H_{22}O_4 + H_2O \rightarrow 2C_6H_{12}O_2.$$

Lusk (2) in 1898 proved that this takes place rapidly in the stomach under the influence of the HCl of the gastric juice.

When the food leaves the stomach, therefore, the carbohydrate is already pretty well digested. The process is continued speedily by the amylase of the pancreatic juice, which would suffice of itself to complete the whole series of changes, if no other enzyme were available.

We have been interested in our laboratory in the enzymes of the intestinal juice produced by the mucosa of the intestine. We have already proved that a piece of intestine transplanted (3) into the abdominal wall under the skin secretes a greater quantity of juice when the dog eats, even when there is no connection by blood vessels or nerves with the rest of the alimentary tract. Incidentally, this proves hormone control of this secretion. Two of the enzymes which have been studied are an intestinal amylase and a sucrase. The action is feeble compared with the enzyme of the pancreas, nevertheless they are sufficient to completely split several grams of starch and cane sugar in the course of 24 hours.

CARBOHYDRATE IN THE BLOOD.

It is clear then that we are specially equipped with a very efficient series of chemical agents to digest carbohydrates speedily—far more speedily than either fats or proteins can be digested. When digestion is complete, the starch is all reduced to glucose and the several disaccharides have been reduced to monosaccharides. Those which are not glucose, like fructose

and galactose, get transformed into glucose either in the course of absorption or in the liver, so that all the carbohydrate is glucose by the time it reaches the general circulation for distribution to all the tissues.

This sugar of the blood may be formed also from protein as was first proved by Cl. Bernard, the great French physiologist, who flourished about the middle of the last century. He was following the fate of foodstuffs which disappeared from the alimentary tract and had reached the point of investigating what happens in the liver which, you remember, receives all the blood coming from the alimentary organs through the so-called portal system. Taking blood as it entered the liver by the portal and comparing it with blood as it leaves the hepatic vein, Bernard found that when a dog had been fed a large amount of meat the liver gave up far more sugar than it received without any change in the amount retained. This could only mean that carbohydrate is formed from protein. We know now that the reaction fundamentally is as follows:

$$CH_3 \cdot CH\ HN_2 \cdot COOH + H_2O = CH_3 \cdot CHOH \cdot COOH + NH_3$$
$$2\ CH_3 \cdot CHOH \cdot COOH = C_6H_{12}O_6.$$

Alanin, a 3-carbon amino acid typical of the building stones in protein is hydrolyzed to lactic acid and from two molecules of this one of glucose can be formed. Another possible reaction is by oxidation of pyruvic acid and by reduction to glucose.

We have then at least two sources of the sugar in the blood—the carbohydrate of the food and the protein not needed for growth or repair of the tissues. The capacity of the body to handle sugar may be determined by what is called a tolerance test. Injecting a known amount of glucose at a certain rate into a vein it very quickly mixes with the whole blood and the level of blood sugar found in any vein plotted against time gives a tolerance curve. If the curve falls promptly tolerance is good; if it falls slowly, tolerance is poor. A few curves obtained on (4) normal and diabetic dogs are given later (p. 349).

The whole picture of sugar regulation in the blood is nicely illustrated by a diagram designed by Drs. Ringer and Baumann (5) for their article in Endocrinology and Metabolism, published in 1922. It shows the ways in and the ways out, and the different organs which participate in maintaining the blood sugar at an average normal level. This chart, however, requires a little modification now to express what has been learned regarding the action of insulin and epinephrin.

METABOLISM OF CARBOHYDRATE.

There are three ways in which sugar of the blood may be disposed of normally. It may be oxidized at once to yield physiological energy and heat; it may be converted to glycogen for temporary storage—a checking account in the bank; or it may be converted to fat—a savings account or permanent investment. The chief tissue for oxidation is muscle, although practically every other tissue, so far as they have been studied, can also oxidize sugar. This universal capacity to burn this foodstuff has lead some authors, rather hastily as it seems to me, to conclude that sugar is the only fuel of life. This conception received great encouragement a few years ago by the development of the so-called Hill-Meyerhof theory of muscular contraction and recovery. Hill (6) found that when a frog's muscle contracts in oxygen it gives off a little heat during the contraction phase, but gives off much more during recovery after the contraction and relaxation have passed. When the muscle contracts in nitrogen, only the first or "initial" heat is produced, the delayed or recovery heat is wholly lacking. It happens that this fits in perfectly with observations previously made by Fletcher and Hopkins that when a muscle contracts it produces lactic acid, the lactic acid disappearing rapidly if oxygen is supplied, but not disappearing if the muscle is bathed by nitrogen gas. Hill inferred that it was the cleavage of some carbohydrate into lactic acid which produced the initial heat and the oxidation of lactic acid which produced the heat of recovery. Meyerhof (7) working on the same subject by biochemical methods came to the conclusion that the only carbohydrate which can fit the requirements is glycogen, which is a normal constituent of all muscle. He agreed with Hill that the cleavage produces lactic acid, and in fact that the yield of heat which Hill found is just the theoretical heat which should appear when glycogen is broken down to lactic acid, plus the amount used in deionizing the protein combined in solution with alkali and making of it a lactate salt. Meyerhof's equation for the process was as follows:

$$5/n\ (C_6H_{10}O_5) + 5H_2O \rightarrow 5C_6H_{12}O_6 \rightarrow 1C_6H_{12}O_6 + 8\ C_3H_6O_3 \text{ (naerobic phase)}$$

$$1C_6H_{12}O_6 + 8C_3H_6O_3 + 6\ O_2 \rightarrow 6\ CO_2 + 6H_2O + 4C_6H_{12}O_6 \rightarrow 6CO_2 + 10H_2O + 4/n\ (C_6H_{10}O_5).\quad R.\ Q. = 1.0$$
(oxidative phase)

The second part—the oxidative phase—describes what happens when plenty of oxygen is present. You will observe that we start with 5 molecules of glycogen and end up with 4. Differently expressed, we start with 30 carbons as glycogen and at the end of the first reaction we have 30, 6 of them as glucose and 24 as lactic acid. In the second reaction the glucose gets oxidized and the 24 carbons of lactic acid (8 molecules) get resynthesized again to glycogen. In Meyerhof's conception the oxidation of lactic acid is for the purpose of restoring the potential energy of glycogen just as if a spring were being wound up or, still better, as if a battery were being recharged ready to deliver its energy again when the switch is thrown, i. e., when the muscle gets a stimulus from a nerve. In his original statement Meyerhof implied clearly that only carbohydrate could be oxidized to furnish the energy for synthesis. Later he disclaimed any intention of excluding fat as a source of this energy.

However, this beautiful conception has in the last six years been considerably defaced. You will observe that the star performer in that view is lactic acid. What Hill (8) calls the "revolution in muscle physiology" which broke out on the last day of 1926, brings forward an entirely new star performer which is called "phosphagen," a compound of creatin and phosphoric acid. It turns out on Hill's own confession that he and Meyerhoff had compromised a small error in their results for the sake of the major conception and in the hope that future work would clear up the discrepancy. The Eggletons (9) in England described phosphagen and almost simultaneously Fiske and Subarrow (10) at Harvard described the same compound as phosphocreatine, showing that it is a very labile substance yielding equivalent proportions of creatine and phosphoric. The Eggletons showed further that this substance is broken down when muscle contracts and disappears again when oxygen is admitted. Hill's own work with Hartree had revealed that there is a small amount of the delayed heat not accounted for by the oxidation of lactic acid and Embden and co-workers proved that some lactic acid appears after contraction is over. These facts did not fit into the nicely balanced thermodynamic equation of Hill and Meyerhof. There were several other facts which we shall not have time to enumerate that were not easily reconciled with the theory. The final blow came, however, with the accidental discovery by Lunds-

gaard (11) at Copenhagen in 1930 that muscle poisoned with iodoacetic acid contracts without producing any lactic acid. This has now been fully confirmed many times and the new theory, which must take Lundsgaard's name, includes phosphagen as follows: When a muscle contracts phosphagen breaks down into creatine and phosphoric acid. Fischer and Meyerhof (12) and Lundsgaard (13) independently have shown that this furnishes the energy for contraction. The energy for resynthesizing the phosphagen in turn comes in part from the cleavage of glycogen to lactic acid, which explains why lactic acid appears after contraction, and the energy for rebuilding the glycogen comes from combustion of *lactic acid or glucose*, or, as many now believe, from fat if carbohydrate is not present in sufficient quantity. Thus we see how carbohydrate is disposed of in muscular work. Every contraction, however small, takes place by the release of potential energy from a complex compound when it suddenly explodes, so to speak, into smaller fragments. But the mechanism is automatic. No sooner is this compound exploded, than the fragments are gathered together and resynthesized in part, by expenditure of energy from another explosion—this time of glycogen, a complex carbohydrate. The second explosion is unexploded, if I may coin the word, by combustion of other carbohydrate and lactic acid, a degenerate form of sugar, becomes ennobled, so to speak, as it continuously returns to the higher form. Lohmann (14) recently has shown that the formation of lactic acid from glycogen in dialyzed muscle extract requires the action of a co-ferment system consisting of inorganic phosphate, adenyl-pyrophosphate and magnesium. Presumably this system is operative also in the live muscle.

Lactic acid, we know now, can be formed in all kinds of tissues, which leads us to suppose that much the same mechanism for release of energy exists in all. But it does not appear in more than trifling amounts *unless oxygen is lacking*. Any form of asphyxia whether local or general always reveals it in larger amount. When a muscle works faster than the physiological rate, which means faster than oxygen can be supplied to resynthesize the lactic acid, it becomes fatigued, as we all know. Washing away or oxidizing away the lactic acid relieves fatigue.

THE RESPIRATORY QUOTIENT.

Dr. Carpenter has already explained to you what the respiratory quotient signifies. This relationship of the volume of CO_2 eliminated to the volume of O_2 absorbed varies ordinarily only between about 0.73 to 1.00. The lower figure means combustion of nearly pure fat, the higher of nearly pure carbohydrate. These facts follow from the following simple equations:

Tripalmitine is $C_3H_5(C_{16}H_{31}O_2)_3$ or

$$C_{51}H_{98}O_6 + 72.5\ O_2 = 51\ CO_2 + 49\ H_2O$$

$$\frac{51\ CO_2}{72.5\ O_2} = 0.703$$

Glucose

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O.\ \text{Hence}\frac{6\ CO_2}{6\ O_2} = 1.0.$$

It is relatively simple to obtain respiratory quotients on the entire body, and Dr. Carpenter has explained to you the method. Having found the non-protein R. Q., then, for any quotient intermediate between 0.707 and 1.0 we can by a relatively simple calculation find the percentage of the total oxygen taken up by the oxidation of each of the two non-nitrogenous food stuffs. Thus $100\ \frac{\text{R. Q.} - 0.707}{0.293}$ gives the percentage of carbohydrate oxygen; $100\ \frac{1.00 - \text{R. Q.}}{0.293}$ gives the percentage of oxygen for fat.

If we eat large amounts of carbohydrate we are sure to obtain R. Q.'s close to 1.0 and if we continue on such diets for a few days we get R. Q.'s higher than 1.0, which mean that carbohydrate is being converted to fat, thus:

$$4\ C_6H_{12}O_6 + O_2 = C_{16}H_{32}O_2 + 8\ CO_2 + 8H_2O.\ \frac{8\ CO_2}{1\ O_2} = 8.0\ \text{R.Q.}$$

glucose palmitic acid

The R. Q. of this reaction would be 8.0, but of course only a little carbohydrate can be converted to fat at a time, and the consequence is a little of this reaction would be mixed with much of the ordinary metabolism with a quotient say of 0.90.

Suppose 2 per cent of the metabolism represented a R. Q. of 8.0 and 98 per cent represented an R. Q. of 0.90, and suppose the total oxygen absorbed was 10 L. Taking 0.2L of O_2 at an R. Q. of 8.0 the CO_2 would be 1.6 L, and the total CO_2 would be 8.82 + 1.6 = 10.42. Since the O_2 we have supposed is 10 L, the total R. Q. would be 1.04. The highest R. Q.'s ever obtained were about 1.5–1.6 which at the same rate of O_2 absorption we have imagined would mean the use of approximately 10 per cent of the oxygen for the conversion to fat, with a R. Q. of 8.0 and 90 per cent for ordinary metabolism with the R. Q. of 0.90.

The inverse relationship holds if we have conversion of fat to carbohydrate. This is a hotly controverted question at present and we shall encounter some new evidence in the latter part of this lecture. Suppose palmatic acid were converted over to glucose by Bleibtrau's reaction, what would be the R. Q.?

$$3\ (C_{16}H_{32}O_2) + 21\ O_2 = 8\ (C_6H_{12}O_6)$$

There is no R. Q. of this reaction because no CO_2 is produced. What the effect on the total R. Q. would be could only be determined by knowing how much sugar was being formed. Let us suppose that we could demonstrate the production of 10 gm. sugar in a period in which 8 L of CO_2 were being eliminated and the basal R. Q. was 0.8, i. e., 10 L of O_2 were absorbed. Imposing the special conditions which produce 10 grams sugar the extra oxygen necessary is found by the proportion

$$\begin{array}{rrrrr} 8\ (C_6H_{12}O_6) & : 21\ O_2 & : : & 10\ \text{gm.} & : x \\ 1440 & : 672 & : : & 10\ \text{gm.} & : 4.66\ \text{gm.} \end{array}$$

This gives the O_2 in grams. Multiplying by 0.7 (actually 0.6998) we get 3.26 liters. Adding this to 10 gives 13.26 liters and dividing into 8 L CO_2 we arrive at the R. Q. 0.60. Properly obtained quotients in this neighborhood can mean nothing else than the formation in metabolism of oxygen-rich substances (carbohydrates) from oxygen-poor substances (fats).

REGULATION OF CARBOHYDRATE METABOLISM.

In 1889 Minkowski working at Königsberg in East Prussia, observed that a dog whose pancreas had been removed excreted large amounts of sugar. This was the first step toward solution of the extremely important medical problem of diabetes mellitus. There had been many indications that the pancreas, if not the actual site of the disease, at least was involved. For example,

autopsies frequently had revealed that the pancreas was very much shrunken in the last stages of diabetes. There had also been indications of degenerative processes visible under the microscope. Minkowski himself undertook to demonstrate that the pancreas contains an extractable substance which can take the place of the pancreas itself in the regulation of carbohydrate metabolism, for it was just at this time, as Minkowski was continuing his work on pancreatectomy and its effects on metabolism, that Murray in England was demonstrating that an extract of thyroid gland can replace the gland itself in restoring a myxodematous person to normal. Minkowski did not succeed in this attempt for reasons which now are perfectly clear. But he did formulate perfectly definite ideas as to the role played by the pancreas in carbohydrate metabolism. He stated that the reason sugar appears in the urine when the pancreas is removed or when seriously diseased, is that sugar cannot be oxidized. Something produced by the pancreas participates in the oxidation of sugar. When that substance is lacking, oxidation fails, sugar piles up in the blood, and leaks out through the kidney. The name "hormone" for such a material had not yet been invented, but of course now we apply the term hormone to such a product continuously formed by an organ, and delivered directly to the blood stream.

Several observers have now demonstrated that the general seat of the metabolic action of insulin, so far as combustion is concerned, is in the muscles. Taking blood from the arteries supplying the muscles and from the vein draining the blood away, before and after introducing insulin, shows clearly that the difference in sugar concentration between these two bloods increases when insulin reaches the muscle. A second important action, probably not less important than the combusion of sugar, is its prompt conversion to glycogen when excess exists in the blood. This process also is facilitated by the presence of insulin as has been demonstrated clearly by Cori and Cori (15).

As early as 1907 it was surmised by Zuelzer that the internal secretion of the pancreas, which regulates sugar metabolism, is antagonized by epinephrin, the secretion of the adrenal gland. Zuelzer (16) actually had a pancreas preparation which would diminish the excretion of sugar of a diabetic dog and would lower the blood sugar previously raised by the injection of epinephrin. This antagonism between insulin and epinephrin is now well established. When epinephrin is injected into a

normal animal the blood sugar rises because glycogen in the liver is converted into glucose rapidly and passes out into the blood stream. Epinephrin even reduces the combustion of sugar (17). When insulin is given exactly the opposite effects are seen. Blood sugar is reduced by two methods, combustion in the muscles and storage of glycogen particularly in the liver. It has been suggested that an excess production of epinephrin, even though small in amount, continuing over a period of years, might possibly be the cause of diabetes mellitus. Insulin, according to this idea, would be continuously suppressed in its action and eventually stopped altogether. Epinephrin would account for the high blood sugar and the inability of the diabetic organism to oxidize sugar or to form glycogen. This remains only a working hypothesis.

A rival theory to that of Minkowski, originally conceived by vanNoorden in Vienna, is that in the absence of the pancreatic hormone, sugar is produced in excessive quantity by the liver and piles up in the blood, not because it cannot be burned, but merely because more is produced than is required in the muscles and elsewhere. Insulin then would have as its primary function the inhibition of sugar formation. This so-called over-production theory has some few facts in its favor. But the discovery of insulin has tended to confirm Minkowski's idea that the primary function of insulin is oxidation rather than the suppression of sugar formation.

For example, a dog made completely diabetic by extirpation of the pancreas has an R. Q. close to the theoretical for the combustion of fat, namely, 0.7. Quite frequently the quotient falls somewhat below this, indicating, according to one interpretation that fat is being converted to sugar and according to another interpretation that fat is only partially oxidized, the products instead of being excreted through the lung being excreted through the kidney. These products are the so-called acetone bodies, diacetic acid, acetone itself and β-hydroxybutyric acid. I shall not have time to go into the chemistry of these ketones, so called, but will indicate merely that if these partially oxidized products are formed in large quantity, there is no doubt that the result would be a depression of the R. Q. Magnus-Levy has concluded that a normal subject producing 40 grams of Beta-hydroxybutyric acid would have an R. Q. in the neighborhood of 0.68. Giving insulin invariably increases the respiratory quotient, provided glucose is available.

GLUCONEOGENESIS.

I shall devote the remainder of my time to a discussion of the processes described by this term, which means the new formation of sugar from either protein or fat. The term was introduced by the vanNoorden school to account for the excess production of sugar from other than carbohydrate sources. It has been revived and discussed energetically by Macleod and his pupils at Toronto and latterly at Aberdeen. There are three main lines of investigation of this subject: (1) The D:N ratio in the urine of diabetic subjects; (2) Perfusion experiments, chiefly perfusions of the liver; (3) Respiratory metabolism.

The D:N ratio is the relationship of dextrose in the urine of a diabetic animal to the nitrogen excreted. The argument as regards the new formation of sugar will appear in a moment. We have three well studied types of diabetes: (1) the human subject, seriously ill because of degeneration of the pancreas; (2) the animal, chiefly the dog, after removal of the pancreas; and (3) the animal poisoned with the glucoside phlorhizin. In all three of these types of diabetes sugar which cannot be oxidized appears in the urine. When there is no carbohydrate whatsoever in the food the carbohydrate of the body itself is quickly exhausted and nevertheless sugar continues to be excreted. Obviously it must come from some other source than carbohydrate. Now if the excess sugar bears a definite ratio to the nitrogen of the urine, since nitrogen can come only from the protein, it will be safe to infer that the sugar also comes only from the protein. The facts are that the D:N ratio is a reasonably fixed quantity in the phlorhizinized animal, as Graham Lusk was the first to prove definitely. Lusk's ratio, 3.65 to 1, has become a pilot light in the study of diabetes. It means that a dog, under the influence of phlorhizin for about three days, fed meat or meat and fat, will continue to produce sugar in relation to N of the urine in such a proportion that 100 grams of protein is forming approximately 58 grams of sugar. Incidentally, this fact illustrates the extreme difficulty in which the totally diabetic person found himself before the discovery of insulin. He lost through the urine all of the carbohydrate eaten, nearly 60 per cent of his protein, and could very imperfectly metabolize fat. In the depancreatized dog the average D:N ratio beginning about two days after the pancreas was removed, and continuing as long as the dog could

eat a meat or meat and fat diet was, as Minkowski found, about 2.8 to 1. This ratio ever since has been called the Minkowski ratio. It means that approximately 44.8 grams of sugar can come from 100 grams of protein in the depancreatized dog. In our experience, however, the Minkowski ratio is much more difficult to establish than is Lusk's ratio on the phlorhizinized dog. After all, it is only an average ratio of some nine dogs as studied in Minkowski's laboratory and fed exclusively on meat. Ratios ranging between 2.6 and 1.0 are not at all uncommon, when the appetite fails, as frequently happens in the later stages of insulin deficiency. However, the interesting point in this particular connection is that the ratio under meat feeding rarely goes higher than, say, 3.0.

In human diabetes one is met by the difficulty that the patient is not always reliable. You can not control your experiment so well as you can with an animal. You cannot lock up a diabetic patient in a cage and have the cage locked in a room and see that the patient gets only certain items of food and that no urine is lost. As a consequence, ratios have been much more variable in human diabetes than in animal experimentation.

If all of the carbon of protein were converted to sugar, the D:N ratio would lie from 6.4 to 6.7, depending upon the exact composition of the protein fed. To prove beyond a doubt the conversion of fat to carbohydrate, it is conceded by the believers in gluconeogenesis from fat that the ratio must be higher than 6.7 to 1. Such ratios have been reliably reported in clinical studies of human diabetes, but it must be said that in the most carefully controlled work, as, for example, in Woodyatt's laboratory in Chicago, in Wilder's laboratory at the Mayo Clinic and in Joslin's laboratory in Boston, such ratios are very infrequently found. Likewise ratios of this order are never found in properly controlled work on diabetic animals. Therefore the evidence from the study of the D:N ratio is that sugar cannot be formed from fat. The fundamental economy underlying this fact seems to be the following: Nature is a better banker than man. Whenever we have an income of energy greater than our expense requirements, the balance is put into savings. Normally we have a checking account which we call glycogen and we have a more permanent savings account which we call fat. Now it is obviously in the interest of good physiological economy, as it is in the interest of good

financial economy, to conserve the more fixed savings. It should be, and it is easier to convert a favorable balance into savings than to convert permanent savings back into small change for current expense account. In primitive man this physiological principle had survival value, for when food was abundant the surplus energy could be stored away in his body as fat and could be drawn upon with difficulty only, inducing him thereby to seek other sources of food before the reserve had been exhausted. This principle is by no means so important for modern man with food everywhere abundant. It is rather an embarrassment because we find it all too easy to put food into the reserve account and all too difficult to take it out again.

The second line of evidence in this study of gluconeogenesis comes from the perfusion of the liver. This is a very common type of physiological experiment. Much information has been gained regarding the intermediary stages of the metabolism of protein and carbohydrate and to a less extent of fat by setting up an apparatus by which defibrinated blood can be pumped through the blood vessels of the liver over and over again, the liver meantime being kept at body temperature in a water bath and as nearly normal as we can imitate normal conditions outside the body. For example, it was long ago discovered that lactic acid in the perfused liver could be converted to sugar and also to glycogen. This supplements the fundamental muscle physiology which we were discussing a while ago. Also, it was learned by perfusion of the liver that the amino acids can form sugar. In recent years this type of experiment has been used in the attempt to show that fats passed through the liver will yield sugar. One such set of experiments performed in Dale's laboratory in London, purported to show that when ordinary defibrinated blood was passed through the liver of a dog or cat whose liver glycogen had been reduced to a minimum by feeding the animal for several weeks on a high fat diet, more sugar came out of the liver than could be accounted for by the small amount of glycogen remaining or by the protein metabolism of the liver. The inference was that the excess sugar was coming from fat. These experiments published by Burn and Marks (18) were repeated in our laboratory by Dr. Gregg (19) with more careful attention to several details than Burn and Marks had applied to the experiments. For example, Burn and Marks claimed that the dis-

tribution of glycogen remaining in such a liver was perfectly uniform from lobe to lobe. Gregg found that this was not the case. Secondly, they overlooked the fact that much of the reducing substance of the blood is not sugar at all, but is uric acid, creatinine, glutathione, ergothionine, etc. In other words, they failed to determine the true or fermentable sugar. Gregg supplied this defect and he found that the true sugar coming from the liver not only did not increase, but actually decreased in a considerable majority of his experiments. It was necessary, Gregg found, to set up real carbohydrate and fat balances from the beginning to the end of the experiment. Such balances are illustrated in the accompanying table (Table I).

TABLE I.

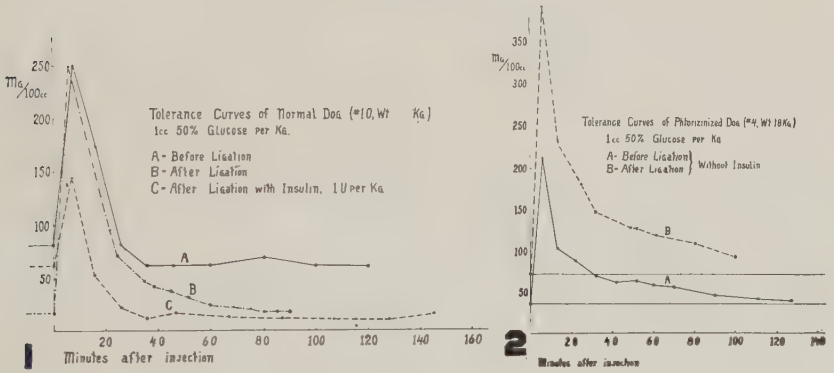
CARBOHYDRATE AND FAT BALANCE IN LIVER PERFUSION.

TOTAL WEIGHTS	BEGINNING		END	
	Carboh. gm.	Lipids gm.	Carboh. gm.	Lipids gm.
Blood sugar	0.644	.....	0.568	.....
Blood sugar removed	.....	.....	0.246	.....
Free sugar	0.206	.....	0.050	.....
Glycogen as free sugar	0.330	.....	0.183	.....
Liver lipids	.....	4.740	.....	3.670
Blood lipids	.....	0.567	.....	0.400
Blood lipids removed	.....	.....	.....	0.189
Total grams	1.180	5.307	1.047	4.259

More recently Jost (20) has claimed that perfusion of the liver with phospholipids yields sugar which can only be accounted for on the assumption that these phospholipids are converted over to carbohydrate. I have no means of judging the accuracy of this work. However, some other experiments reported from Best's laboratory in Toronto, which claim to prove that lecithin fed to diabetic animals causes the production of extra sugar in the urine, have been checked in our laboratory in a few experiments and have been found incorrect. When carefully purified lecithin is fed to a diabetic animal, it does not affect the D:N ratio or the R. Q. It is quite possible that this type of experiment will eventually furnish convincing evidence that sugar may be produced from fat. In our laboratory Dr. Eaton and I have found that a fraction of the fat extractable from the

pancreas and belonging to the cerebrosides (or possibly sphyngomyeline), depresses the R. Q. and increases the D:N ratio of depancreatized dogs (21).

The change in sugar tolerance when an animal is made diabetic with phlorhizin is shown in Charts 1 and 2, taken from a recent publication on this subject from the Rochester laboratory (4). When a certain definite doze of glucose was injected into a vein of a normal dog and a sample of blood drawn from another vein at frequent intervals, "tolerance curves" like those in Chart 1 were obtained. These could be repeated at three-hour intervals. When insulin was given with the glucose a lower curve (higher tolerance) was obtained (curve C). In the phlorhizinized dog (Chart 2) the tolerance


Tolerance Curves of Normal Dog (#10, Wt Kg.)
1cc 50% Glucose per Kg.
A- Before Ligation
B- After Ligation
C- After Ligation with Insulin, 1 U per Kg.
Minutes after injection
Tolerance Curves of Phlorizinized Dog (#4, Wt 18Kg.)
1cc 50% Glucose per Kg.
A- Before Ligation
B- After Ligation
Without Insulin

curve from the same dose of glucose per kilogram of body weight gave a lower curve (A). This does not indicate higher tolerance; it only shows that some of the sugar passed out through the kidney. When the kidney blood vessels were ligated so that no sugar could escape from the blood to the urine, the dose of glucose produced a much higher curve. The lower tolerance of the diabetic organism is shown by the difference between this curve and a similar test (after ligation of the vessels) in the normal dog (Chart 1). The lower tolerance of the phlorhizinized dog is shown especially by the prolongation of curves A and B in Chart 2 and is due probably to injury to vessels of the liver, similar to the injury produced in the kidney.

The study of the respiratory metabolism of diabetic animals has in the main confirmed the study of D:N ratios. The verdict to date has been that there is no diminution of the R. Q. to the point where one could safely infer that sugar is being

formed from fat. I know of only a few experiments, including one from Boothby's laboratory, which furnished R. Q.'s too low to be accounted for on any other hypothesis. Wilder, Boothby and Beeler (22) were studying quite exhaustively a single case, and they were testing out the specific dynamic action of high fat meals on the diabetic subject. In a few instances they obtained quotients immediately following the meal as low as 0.62 or 0.61. The quotients gradually rose from that level to the level prevailing before the meal, namely, about 0.69. Boothby did not comment on the significance of this quotient in reporting the work. Likewise in Joslin's laboratory in a few cases out of a large number which he studied following meals containing a considerable amount of fat, quotients as low as 0.57, 0.58 and 0.62 were found. Joslin believed these quotients were really significant of gluconeogenesis from fat. Magnus-Levy has stated that in diabetes a quotient as low as 0.65 cannot be explained in any other way and he says a quotient of 0.60 would prove it beyond peradventure. But such a quotient would be obtained in the diabetic only if in the metabolism of 200 grams of protein and 250 grams of fat 350 grams of sugar were formed besides 40 grams of Beta-hydroxybutyric acid.

The main difficulty which stands in the way of getting crucial evidence concerning the conversion of fat to carbohydrate by the R. Q. method is that, if combustion of carbohydrate keeps up with its formation, there is no lowering of the R. Q. For example, if the palmitic acid should be converted to glucose with an R. Q. of 0.60, as described above (page 342), and should then be immediately burned with an R. Q. of 1.0 the quotient obtained would be somewhere between these two values and the low R. Q. would entirely disappear. It occurred to us that the fatty seeds during germination, where low R. Q.'s have frequently been reported and have been interpreted to mean that the fat is being converted to sugar for later conversion to cellulose or combustion would be suitable objects in which to separate the two processes: (1) conversion of the fat to carbohydrate and (2) the combustion of the carbohydrate. The castor bean was chosen and its respiratory metabolism at various stages during germination was studied in the so-called Warburg respirometer (23). It was soon found in confirmation of earlier work on this seed, that after the first few hours of germination low R. Q.'s are regularly obtained.

The table shows some of these quotients obtained on individual beans (Table II). It will be observed that the level of the quotients depends to some extent upon the stage of growth reached. The next question was to study on the whole young plant separated from the endosperm of the seed and to compare its respiratory metabolism with that of the whole germinating bean. The next table shows that the bean as a whole gives as usual its low R. Q., but that when the cotyledons are separated out of the bean and together with the hypocotyl or radicle are

TABLE II.

Castor Bean Experiments.

Single Bean in Warburg App.
Variation with Stage.

Bean No.	Stage of growth L.of hypocotyl mm.	Length of exper. min.	Resp. Exch.		R. Q.
			CO_2 cu. mm.	O_2 cu. mm.	
13.....	12	69.0	234.9	345.6	0.679
14.....	12+	46.5	155.8	231.4	0.535
17 (1).....	16	30.0	208.2	337.8	0.617
18 (2).....	16	32.0	211.4	407.8	0.518
16 (1).....	19	32.0	142.6	247.6	0.576
16 (2).....	19	32.0	130.0	223.3	0.582
					Av.0.585
18.....	20	15.0	83.4	252.9	0.337
12 (1).....	23	34.5	117.3	406.3	0.289
12 (2).....	23	23.0	107.8	287.4	0.373
12 (3).....	23	22.5	98.0	320.4	0.306
10.....	24	31.5	146.7	413.8	0.355
11.....	29	32.5	127.9	496.9	0.257
19.....	32	41.0	160.6	420.9	0.382
20.....	33	37.5	184.0	481.2	0.382
			Av.20-33 mm		0.323

studied in the respirometer, quotients approaching those for combustion of carbohydrate alone are obtained. (Table III.) Comparing now the respiratory metabolism of the endosperm by itself with that of the whole bean, we find that its R. Q. instead of being slightly lower as we expected, is slightly higher. This is probably due to the fact that it is necessary to strip off the seed coat and thereby to expose the endosperm tissue more completely to the outside air, and this results in combustion of some of the already formed carbohydrate. Comparing these two, however, with that of the whole plant, we

find the same contrast as before. Finally, we studied the respiratory metabolism of a considerable number of beans by confining them for several days in a glass bottle and analyzing the air contained in the bottle at the termination of this period. From such studies we learned that the low R. Q.'s prevail not merely over the few minutes necessary to obtain the R. Q. on the individual seed, but throughout the entire germination period. We have here then a demonstration that the two processes can be studied separately—the conversion of fat to sugar producing a low quotient, and the young plant then oxidizing a portion of this sugar and giving a high quotient.

TABLE III.

Respiratory Exchange of Whole Bean, Endosperm and New Plant.

No.	Part	Stage	Length of exper., min.	CO_2 cu. mm.	O_2 cu. mm.	R. Q.
32	Whole bean	40 mm., 1 branch	59.5	236.2	612.2	0.386
32	Endosperm	40 mm., 1 branch	52.0	195.9	426.8	0.459
32	New plant	40 mm., 1 branch	72.0	155.8	140.8	1.106
33	Whole bean	45 mm., 4 branches	57.0	292.9	604.5	0.484
33	Endosperm	45 mm., 4 branches	44.5	184.0	331.7	0.555
33	New plant	45 mm., 4 branches	70.0	173.3	192.5	0.90

The conversion of fat to carbohydrate in these beans has been confirmed by two other methods. Chemical analyses which Dr. H. B. Pierce has carried out in our laboratory demonstrate that the percentage of fat steadily falls from the time germination begins to the end, that the percentage of protein scarcely changes at all, but that the percentage of sugar identified as cane sugar, increases, as does also the percentage of crude fiber or cellulose. The other method consists of burning the entire bean at different stages in a modified oxy-calorimeter. This instrument was devised by Benedict and Fox at Boston for the purpose of studying the heat value of mixtures of food-stuffs when burned in pure oxygen. This was modified sufficiently to collect and weigh the CO_2 formed, to keep the temperature down by cooling the air, and by using a more accurate spirometer so as to measure the oxygen consumed. Dr. Daggs in our laboratory has used this apparatus to burn the ungerminated bean and the bean germinating at several distinct stages up to a total length of the hypocotyl of about

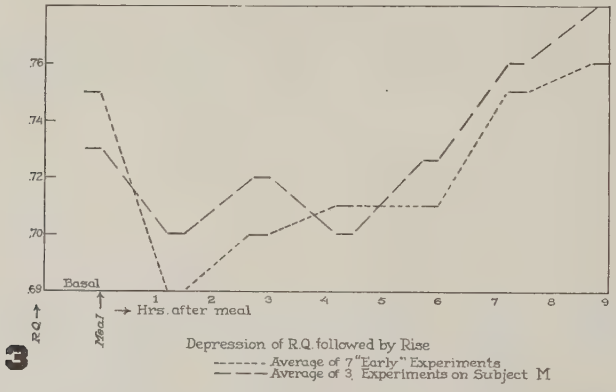
3 inches. The R. Q. increases steadily from the ungerminated stage up to the last stage studied (24).

We have thus obtained a complete demonstration of the conversion of fat to carbohydrate and the partial oxidation of the carbohydrate with the characteristic effects on the respiratory metabolism.

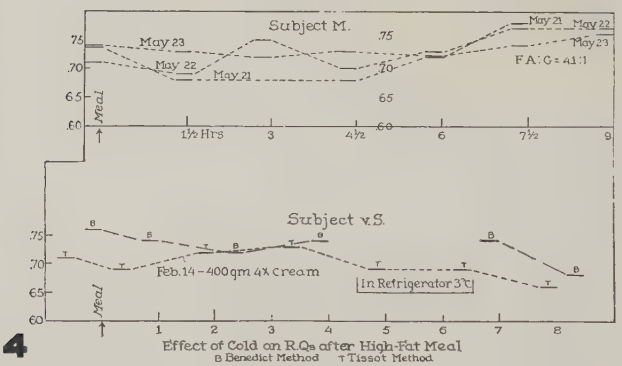
With Drs. Estelle E. Hawley and Carroll M. Johnston (25) we have studied the problem in human subjects. It happened that one of them had an unusually high tolerance for fat. It occurred to us that in such an individual by prolonging a tolerably high fat diet we might deprive him of glycogen sufficiently so that when he was given a large meal of butterfat, selected because it is already emulsified in the form of cream, and therefore quickly digested, the earlier quotients after the meal would show a considerable depression of the quotient, signifying conversion of some of the fat to sugar and its storage as glycogen. Later this glycogen would be mobilized and burned and as a consequence the R. Q. would rise. This hypothesis was predicated on two other demonstrated facts: (1) That at best it is difficult to convert fat to carbohydrate, and (2) that the demand for glycogen as tissue reserve at times dominates the demand for sugar as fuel. Hence to exhibit the conversion, it would be necessary to establish these special conditions: (1) Just that degree of tissue hunger for glycogen which would cause retention of any sugar formed; (2) that there should be no other adequate source of sugar available, and (3) that the subject at the moment of test would be already accustomed to digesting and absorbing large amounts of fat. With these conditions rightly adjusted, the hypothesis was that a certain sequence of R. Q.'s would be obtained that could not be plausibly explained in any other way. The low quotient immediately following a high fat meal when averaged with the high quotient later in the day, would produce a quotient which would stand near the accepted level for combustion of the ingested fat.

Dr. Gregg, in our laboratory, had calculated that the theoretical quotients for butter fat should be 0.72, instead of the usually accepted 0.707 for mixed food or body fats, the higher level being explained by the presence of lower fatty acids in butter which give higher quotients than the usual food fats. Encouragement was obtained from the earlier experiments on this subject and then other subjects, to a total number

of seven, were studied on the same high fat diet. The last diet consisted exclusively of 4X cream, actually 37½ per cent fat by analysis. Only this diet of cream gave the best results (from our point of view. The charts 3 and 4 show that the predicted sequence of quotients could be obtained in single experiments or as an average of several experiments on the same subject or several experiments on different subjects. They are best



3 Depression of R.Q. followed by Rise
------ Average of 7 "Early" Experiments
— — — Average of 3 Experiments on Subject M



4 Effect of Cold on R.Qs after High-Fat Meal
B Benedict Method T Tissot Method

obtained, however, while the subject is still somewhat unaccustomed to the high fat diet. This was not anticipated. The phenomenon of adaptation or improved tolerance to high fat diet has only been observed so far as we are aware by Wigglesworth (26), who observed it in rats. It shows up clearly at several points in our experiments.

The best results, from the standpoint of the hypothesis, were obtained before this improved tolerance had asserted itself. One of the charts shows the improving tolerance in

three successive days on the same subject (M). We call attention to the fact also that sometimes these low quotients continue throughout the day, more especially in subject H, who had a very low tolerance for fat, both in the sense of being unable to oxidize the fat successfully and in the sense of showing the highest ketosis of any of the subjects. In the attempt to explain these low R. Q.'s we have calculated the theoretical depression of the quotient which might conceivably be produced by conversion of protein to sugar. The method involves a rather long calculation which I shall not have time to give in detail. It is a well-established method first introduced by Magnus-Levy and used subsequently by Lusk, Geelmuyden, Macleod, and others. In our experiment if we assume that 58 per cent of the protein metabolized were converted to sugar and stored as glycogen, there would be as a maximum a reduction of the quotient, amounting to 0.025. Secondly, we have undertaken to determine how much the quotient might be lowered by conversion of the glycerol of fat to sugar. It is well known that in the diabetic organism when glycerol is fed, extra sugar appears in the urine equivalent to the amount of sugar which can be formed if all of the carbon of the glycerol were converted to glucose. Making this calculation in our experiments we found that if all the glycerol of the fat meal were converted to sugar, and stored as glycogen, the quotient might be depressed as much as 0.03 and we have some experiments in which the theoretical amount of glycerol was fed on one of the days adjacent to the all cream diet. There was at times a similar drop in R. Q.'s immediately after the meal following glycerol as following the high fat, which would lead one to think that on the day of high fat ingestion all of the glycerol separated from the fatty acids and was converted to glycogen. It is perfectly easy to imagine that this happens when nothing but glycerol is fed, but the total amount of glycerol of the fat cannot possibly be separated from the fatty acid after absorption into the circulation unless the fatty acids at the same time were metabolized. Hence we are limited in calculating the glycerol available for conversion to sugar and thence to glycogen to that amount which corresponds to the highest fat metabolism of the hour when the R. Q. was determined. The highest fat metabolism in a single hour in any of these experiments was 10 grams of the butter fat. The glycerol is roughly one-tenth of this, or one gram. Conversion

of one gram of glycerol to glucose would not lower the quotient more than 0.003.

Next we attempted to correlate the R. Q.'s with the ketosis developed in these subjects. The acetone bodies were determined in the blood and also in the urine. If the ketosis were responsible for the low R. Q.'s as Shaffer has demonstrated should be the case from his *in vitro* work, the quotients should be lowest when the ketosis is highest, and *vice versa*. Our series show, however, that this is not the case whether we consider the total acetone of the blood or of the urine. Finally, we have calculated what would be the effect on the R. Q. from the increase in the ketone bodies in the blood from one period to the next.

It is necessary also to make allowance for the increased ammonia production which results from the acidosis. Making these calculations in several of our typical experiments, we found that the only effect is a lowering of the quotient of not more than 0.01. Now if we imagine that all of these various influences known to have the effect of lowering the R. Q. were operative at once, which in itself is very improbable, we might have a total effect of about 0.04. In other words, a quotient which would be about 0.73, if the fat were oxidized completely as fast as it was absorbed, would be depressed by these several factors to a level not lower than about 0.69. This is the familiar R. Q. of total diabetes.

In this work we have literally scores of quotients lower than this. A considerable number in the neighborhood of 0.60 to 0.65, still more from 0.65 to 0.69. As we have said before, these quotients below 0.69 occur most frequently in the person with a low tolerance or in the very early experiments on other subjects which had a normal or high tolerance for fat.

There is one other possible explanation of low quotients which should be considered before we infer that we have demonstrated in these experiments the formation of sugar from fat. You will remember that the most plausible theory for the oxidation of fatty chains in the body is Knoop's theory of Beta-oxidation. Suppose we have a fatty acid like oleic acid with 18 carbons, and with a double bond between the 9th and 10th carbon. It is assumed that the chain first breaks at this weak point in the double bond and two chains of 9 carbons each are obtained which may be oxidized at either end. Now, if we try to imagine an orderly process of Beta-oxidation, that is, splitting off of two carbons at a time with subsequent formation of CO_2

and water by way of several intermediary products, it is clearly possible that the absorption of oxygen may considerably outrun the formation of CO_2. We believe it is necessary to rule out this possibility before concluding that in the human subject, on a high cream diet, the conversion of fat to carbohydrate has been demonstrated. Furthermore, it will be necessary to find the carbohydrate.

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Fighting the Insects, by L. O. Howard. 233 pp. New York, The MacMillan Co. 1933.

FAT METABOLISM

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According to Voit, a man ingests as a part of his food an average of 56 grams of fat per day. Assuming this average to be correct and allowing for a lower consumption during infancy and childhood, we find that when a man reaches the age of forty he will have eaten approximately 700 kg. of fat, the energy value of which is nearly 6½ millions of large calories. This is equivalent to approximately 2.7×10^{16} ergs or 2.6×10^{9} kilogram-meters. It is, however, less than 20% of the total energy intake.

The tissues of an adult human contain relatively constant amounts of protein and carbohydrate. Their fat content, on the other hand, may vary within wide limits depending upon many factors.

Two main classes of fatty substances are found in animal tissues. Under the French classification these have been designated as the 'element variable' which is made up of true fats and oils, chemically glycerides of fatty acids, and the 'element constant,' consisting of such complex compounds as lecithin, cerebrosides, lipo-proteins and the like. The amounts of the former class of compounds found in the tissues vary widely with different individuals; in starvation these compounds are readily mobilized and used as sources of energy. Substances in the latter class occur in relatively constant amounts in tissues; in starvation they resist the forces of mobilization. From a functional standpoint, the latter are undoubtedly far the more important.

The most characteristic part of the lipid (or fat) molecule is its fatty acid. In order to show more clearly the numerous fatty acids which occur in the food, most of which may enter the body tissues if this food is eaten when body fat is being stored, there is grouped in Table I a list of the "food fatty acids."

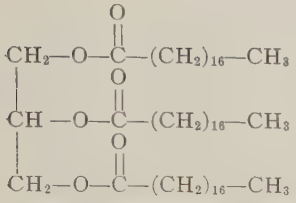
TABLE I.

THE FATTY ACIDS WHICH OCCUR IN FOODS.

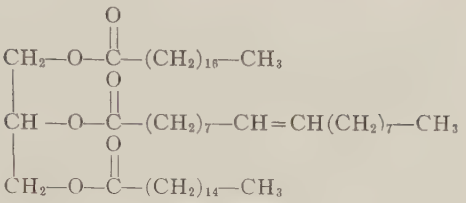
Name of Acid	Formula	No. of HC=CH	Mol. Wt.	Iodine No.	Typical Occurrence in Foods
Butyric	$C_4H_8O_2$	0	88	0	Butter
Caproic	$C_6H_{12}O_2$	0	116	0	Butter
Caprylic	$C_8H_{16}O_2$	0	144	0	Butter, cocoanut oil
Capric	$C_{10}H_{20}O_2$	0	172	0	Butter, cocoanut oil
Lauric	$C_{12}H_{24}O_2$	0	200	0	Butter, cocoanut oil
Myristic	$C_{14}H_{28}O_2$	0	228	0	Butter, nutmeg fat
Tetradecenoic	$C_{14}H_{26}O_2$	1	226	112	Fish oils
Palmitic	$C_{16}H_{32}O_2$	0	256	0	All fats and oils
Hexadecenoic	$C_{16}H_{30}O_2$	1	254	100	Peanut oil, fish oils
Hexadecatrienoic	$C_{16}H_{26}O_2$	3	250	305	Fish oils
Stearic	$C_{18}H_{36}O_2$	0	284	0	Tallow, lard
Oleic	$C_{18}H_{34}O_2$	1	282	90	All fats and oils
Linolic	$C_{18}H_{32}O_2$	2	280	181	Animal lipids, semi-drying oils
Linolenic	$C_{18}H_{30}O_2$	3	278	274	Rarely in foods
Clupanodonic	$C_{18}H_{28}O_2$	4	276	368	Fish oils
Arachidic	$C_{20}H_{40}O_2$	0	312	0	Peanut oil
Gadoleic	$C_{20}H_{38}O_2$	1	310	82	Fish oils
Arachidonic	$C_{20}H_{32}O_2$	4	304	334	Animal lipids, fish oils
Eicosapentenoic	$C_{20}H_{30}O_2$	5	302	420	Fish oils
Docosatetrenoic	$C_{22}H_{36}O_2$	4	332	306	Brain lipids, fish oils
Docosapentenoic*	$C_{22}H_{34}O_2$	5	330	385	Brain lipids, fish oils
Docosahexenoic	$C_{22}H_{32}O_2$	6	328	464	Fish oils
Tetracosanoic	$C_{24}H_{48}O_2$	0	368	0	Brain lipids

*Tsujimoto prefers to designate this acid as clupanodonic (13).

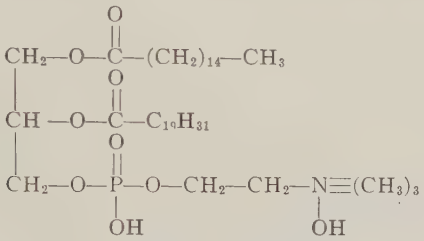
One interesting observation that may be made concerning these fatty acids is that each of them has an even number of carbon atoms. Further, all members of the series of saturated acids from four to twenty-four carbon atoms occur. The unsaturated acids include those of fourteen to twenty-two carbon atoms and of one to six double bonds. The formulae of a number of typical lipids follow:



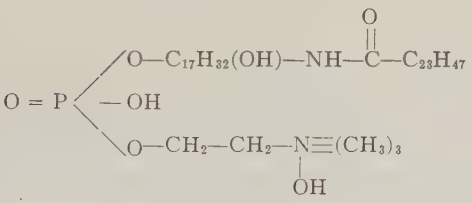
TRISTEARIN.



PALMITO-OLEO-STEARIN.



PALMITYL-ARACHIDONYL-LECITHIN.



SPHINGOMYELIN.

Although there are more than twenty fatty acids occurring in the foods we eat, the common animal body fats are composed chiefly of the glycerides of palmitic, stearic and oleic acids, and in addition generally small amounts of myristic and linolic acids; and less frequently traces of arachidonic. The animal phosphatides usually contain relatively large amounts of more highly unsaturated acids, such as arachidonic. The special function of these phosphatides may be connected in some way with their high unsaturation.

It is worth while to note here two fats which are exceptionally complex—butter fat, which contains at least eleven fatty acids, mostly saturated, and the fish oils which contain even a larger number of acids, most of them with three or more double bonds. Many speculations have been made in unsuccessful attempts to explain the complexity of these fats.

The vegetable fats and oils which are ordinarily used as foods are composed chiefly of palmitic, stearic, oleic and linolic acids. Vegetable foods are relatively low in phospho-lipids.

DIGESTION OF FATS.

Digestion of fats consists of adding the elements of water to form glycerol and fatty acids. This process begins to a very minor extent in the stomach. It is now generally recognized that a fat-splitting enzyme occurs in the gastric juice (1). Although the strong acidity and other conditions in the stomach are not favorable to the action of lipase, there does occur a certain degree of hydrolysis, especially if the fat reaches the stomach in an emulsified condition. As the food passes into the duodenum, however, the conditions change rapidly to those more favorable for saponification. The mildly alkaline juices of the intestine and pancreas and the bile rapidly neutralize the hydrocholric acid. The bile furnishes the sodium salts of glycocholic and taurocholic acid which facilitate emulsification. The intestine and pancreas secrete lipases which act rapidly on the fat as it becomes emulsified. In a relatively short time, therefore, the food fats are changed into glycerol and fatty acids. In the older treatises on digestion this reaction was explained on the basis of the formation of soaps. This appeared necessary to account for the fact that the fatty acids, which are insoluble in water, remain in solution in the digestive juices. Such a conclusion has been criticized in view of the observation that the pH of the intestine is perhaps as often below 7.0 as above; in fact it has been reported as low as 6.0. Theoretically, soaps would be completely hydrolysed and could not exist at this pH. This anomaly has been explained by the work of Verzar and Kuthy (2), who found that the bile salts will dissolve fatty acids with the formation of clear solutions even at pH 6.2.

Reference should be made at this point to the so-called "digestibility" of fats. By this is meant the extent to which fat is digested and absorbed. Earlier work had shown that if a given amount of fat were fed, approximately 90–98% of it was absorbed, the remainder being excreted. In such experiments the fecal fat was determined and estimated as unabsorbed fat. Sperry and Bloor (3), however, found that on a fat-free diet lipids continued to be excreted and, further, the character of this excretion was relatively little affected in amount or kind of fatty acids by food fat. Apparently, therefore, the normal intestine secretes certain lipids constantly. Allowing for this excretion one may infer that when any normal amount of fat is eaten, it is nearly quantitatively utilized. Exceptions to this

statement may be observed when unusually hard fats or fatty acids are fed, such as stearin or stearic acid. These do not melt at body temperature and are very imperfectly absorbed.

ABSORPTION OF FATS.

For many years it has been quite generally believed that the chief path of fat absorption was through the lacteals and lymph vessels of the intestine. The bile salts are indispensible carriers in this process. By holding the fatty acids in solution they favor diffusion through the cells and into the lymph vessels. Thence they are transported as neutral fat to the thoracic duct which empties into the left subclavian vein. The bile salts pass again into the liver which resecretes them into the bile. In this way they go through a continuous cycle of activity. Should this cycle be interrupted by obstruction of the bile duct or by artificial drainage of the bile out of the body following operations on the gall bladder, fat absorption fails. Certain definite digestive disorders are then manifested, the most important of which is the appearance of large quantities of fatty acids in the stools.

Although it is generally agreed at present that the principal path of fat absorption, at least up to 60%, is that just described, recent investigations (4) are leading to the belief that most of the remaining 40% passes into the portal blood. An appreciable lipemia has been described in portal blood during active fat absorption.

A number of important facts about the mechanism of fat absorption are now available.

(1) The fatty acids appear as neutral fats in the lymph. This change from the products of saponification in the intestine involves recombination in glyceride form. It is possible that lipases catalyse both processes. In this synthesis the bile salts probably are set free.

(2) Even if fatty acids or ethyl esters are fed, neutral fat appears in the lymph. Glycerol, therefore, is supplied in the process, either by direct synthesis or from the blood.

(3) The character of the fat appearing in the lymph represents an average between the food fat and endogenous fat. In this connection it should be mentioned that if endogenous fatty acids appear here, the 60% which can be recovered from the lymph is composed of fatty acids from the two sources; actually, therefore, less than 60% of the food fat can be recovered in the lymph.

(4) If a very finely divided emulsion of fat and mineral oil is fed, the former is almost quantitatively and selectively absorbed leaving the mineral oil behind to a degree equally quantitative. The process is, therefore, quite a specific one.

The question may well be asked "why are fats hydrolysed in the intestine only to be resynthesized before appearing in the blood?" Of course this can not be answered positively. Leathes (5) has suggested that by this process of recombination glycerides of different structure may be produced—in other words new fats more characteristic of the new organism. May there not be, therefore, some analogy between fat absorption and resynthesis and protein absorption and resynthesis? The building stones which occur in combined form in the foods are thus liberated by digestion and recombined in the new organism to form compounds characteristic of and perhaps essential to that organism.

Although small amounts of free fatty acids may be found in the blood, they occur in this fluid chiefly combined as neutral fat, phospho-lipid and cholesterol esters. Since these are insoluble in water and serum, they are carried, both by serum and cells, as an emulsion of finely divided droplets. During absorption the serum may actually be milky in appearance due to its fat content.

Following absorption fat may pass to the liver or the tissues, depending on whether it has entered the portal or systemic blood. In consequence of this, three well recognized types of transformations may occur: changes in the liver; oxidation; deposition as body fat.

THE ACTION OF LIVER CELLS ON FAT.

The normal liver contains 5% lipids, about evenly distributed as phospho-lipid and neutral fat. In 1909 Leathes and Wedell (6) showed that when cats are fed on certain fats the iodine number of the liver fatty acids is greater than that of the fatty acids of the food. Leathes suggested, on the basis of these and other results, that liver cells have the property of introducing double bonds into (desaturating) fatty acids, thus making them more reactive so that they are more easily oxidized in the tissues. Thus from stearic acid, oleic acid (or iso-oleic) would be formed; from oleic there would result linolic. Space will not permit adequate discussion of this "desaturation theory."

Suffice to say, the phenomenon of increased unsaturation may be explained on the basis of selective deposition of certain highly unsaturated acids which occurred in the oils which Leathes fed. The liver is unusually rich in phospholipids which seem to attract specifically acids of high unsaturation. The validity of the theory has been further criticized since the discovery of arachidonic acid ($C_{20}H_{32}O_2$) in the liver by Hartley (25), subsequently confirmed by Levene and Simms (7), Brown (8), Klenk and Schoenebeck (9) and very recently by Bloor and Snider (10). This highly unsaturated acid of twenty carbon atoms and four double bonds occurs in considerable amount in the liver (as high as 12% of the total fatty acids) (11). If this acid had originated by desaturation of C_{20} fatty acids, one would have to account for these acids in the food. Moreover, by desaturation one would expect to find C_{20} acids of one, two, three and four double bonds; the last, however, is the only one known to occur. Very recent reports (9) are to the effect that under certain conditions C_{22} acids with five bonds occur in the liver. Actually, the content of C_{20} and C_{22} acids in ordinary food fats is quite insignificant. It would appear more reasonable, therefore, to assume that highly unsaturated acids result from some other obscure process, probably synthetic in nature. The writer considers the evidence for desaturation as quite unsatisfactory, although, of course it has not been disproved.

Whether the fatty acids are desaturated in the liver or not, certain observations point to very active fat metabolism in this organ. Experiments have shown that liver fat changes rapidly. In starvation, for example (12), increased amounts of body fat are rapidly mobilized into the liver. Variations in food fat are rapidly reflected in the character of liver fat. Further, in certain pathological conditions such as acute yellow atrophy and miliary tuberculosis the liver may contain as much as 50% of lipids, mostly neutral fat, so that the ratio of neutral fat to phospho-lipids increases from a normal of about 1.0 to one has high as 71 (11). The forces which attract fat to this organ in such large amounts are obscure, although the abnormality may lie not in unusual attraction but in liver hypofunction—a failure of fat to be used in liver tissue. Thus fat may be attracted to liver cells by normal forces; then due to failure of liver function it remains there.

OXIDATION OF FAT.

The fat which has been absorbed into the blood and which may have passed through the liver changed or unchanged is transported to the various tissues where it may be either oxidized or stored according to the balance of supply and demand of energy available and required by the tissue cells. The forces which come into play at this point are entirely obscure; the effect of the glands of internal secretion, however, is recognized.

If fat is oxidized, we do not know as yet whether this happens to the fat molecule as a whole or whether hydrolysis precedes oxidation. In any case it is quite certain that the glycerol follows one course of degradation, and the fatty acids another.

TABLE II.

FATE OF PHENYL FATTY ACIDS FED TO DOGS.

ACID	FORMULA	EXCRETED IN URINE (Conjugated)
Benzoic	C_6H_5COOH	C_6H_5COOH
Phenylacetic	$C_6H_5CH_2COOH$	$C_6H_5CH_2COOH$
Phenylpropionic	$C_6H_5CH_2CH_2COOH$	C_6H_5COOH
Phenylbutyric	$C_6H_5CH_2CH_2CH_2COOH$	$C_6H_5CH_2COOH$
Phenylvaleric	$C_6H_5CH_2CH_2CH_2CH_2COOH$	C_6H_5COOH

(Knoop)

The classic researches of Knoop were the first to give us a clear picture of the mechanism of the oxidation of the fatty acids. From the standpoint of their chemical properties it would be expected that these acids would be attacked either on the alpha carbon atom or at a double bond. Apparently, however, in living cells the point of attack is the beta carbon atom. Knoop (14) fed to dogs phenyl derivatives of the lower fatty acids noting how they were excreted, the results of which are given in Table II.

Benzoic and phenyl acetic acids are excreted in combined form in the urine, both groups being resistant to oxidation. Phenyl propionic and valeric acids are likewise excreted as benzoic, whereas phenyl butyric appears as phenyl acetic. These results support the so-called beta-oxidation theory, which is outlined in Figure 1.

It is apparent from this outline that the normal course of oxidation is a succession of cycles involving the loss of one molecule of acetic acid (two carbon atoms) at the end of each cycle. The final product, therefore, is acetic acid which

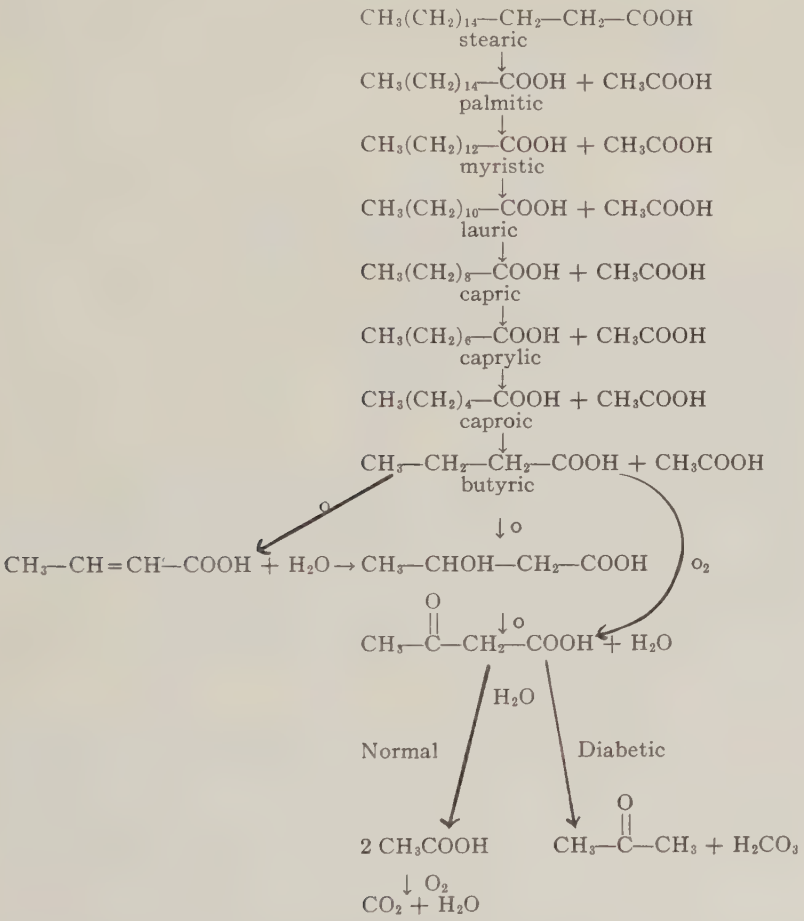


FIGURE 1.

BETA-OXIDATION OF STEARIC ACID.

eventually oxidizes to carbon dioxide and water. It is now well recognized that an intermediate of carbohydrate metabolism is essential to the completion of this process; in its absence, instead of acetic acid, other incomplete oxidation products appear, the so-called acetone bodies. Such a condition exists in diabetes.

This disease apparently results from hypofunction of the pancreas, which fails to supply adequate amounts of insulin. As the supply of insulin diminishes, less and less carbohydrate is oxidized. The substance necessary for final oxidation of the derivatives of butyric acid is no longer available. Ketogenesis results. So long, however, as there is a proper balance between sugar oxidation and fat oxidation (antiketogenesis) butyric acid is converted quantitatively into acetic acid. In the early stages of diabetes there may be enough insulin to prevent ketone formation, but not enough to prevent excretion of sugar in the urine. Ketogenesis appears only in the later and more severe stages of the disease. It is finally accompanied by severe and terminal acidosis. When the acidosis is relieved by administration of glucose and the missing intermediate is supplied by combined administration of glucose and insulin, the situation in most cases is rapidly relieved.

Aside from the evidence furnished by Knoop and by the metabolism in diabetes four other investigations serve to confirm the beta-oxidation theory:

(1) The observation of Dakin (15) that when phenyl propionic acid is fed to dogs, the intermediates predicted from the theory, phenyl-beta-hydroxypropionic acid, benzoyl acetic and acetophenone as well as hippuric acid could be detected in the urine.

(2) Dakin's discovery (16) of the analogous in vitro reaction whereby soaps may be oxidized on the beta-carbon atom by hydrogen peroxide.

(3) Embden and co-workers' experiments (17) showing that when soaps of even carbon acids were perfused through the surviving liver, acetone appeared in the blood; when soaps of odd carbon acids were perfused, no acetone was formed.

(4) The discovery by Kahn (18) that when synthetic fats with an odd number of carbon atoms were fed to diabetics, little or no acetone bodies resulted.

Beta-oxidation explains well the oxidation of the saturated acids, but how about the unsaturated acids? Oleic acid, for example, with a double bond between the ninth and tenth carbon atoms, should be more easily attacked by oxygen than stearic. In the body, however, this apparently is not true. What information is available appears to show that oleic acid, and in fact the other unsaturated acids, likewise oxidize on the beta-carbon atom. Certain of the tissues, such as the liver,

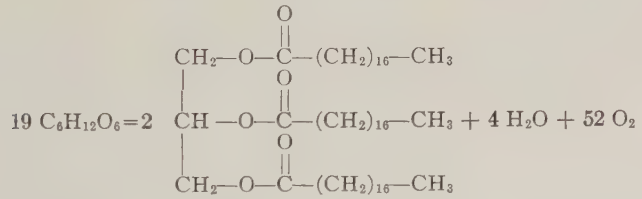
suprarenal, spleen, and brain, concentrate acids with as many as four and five double bonds. Instead of being easily oxidized, these seem to be unusually stable during life.

To summarize the oxidation of fats, therefore, it may be said that these are probably first hydrolysed into glycerol and fatty acids, the former oxidizing similar to the carbohydrates and the latter by series of beta-oxidation cycles. As a summation of this process in the normal individual a gram of average fat yields a little over nine kilogram calories of heat.

THE SYNTHESIS AND STORAGE OF BODY FAT.

We have now to consider the formation and deposition of body fat. If the energy value of the food intake exceeds that required by the body, fat is stored. This may result by synthesis either from preformed fatty acids furnished by the food or by the generation of fatty acids from carbohydrate (and indirectly from protein). Both processes are important although little is known about them. What metabolic force, for example, determines whether a fatty acid molecule is burned or stored? If burned, does this happen in the cells of the liver, in the blood cells, or in certain tissue cells? How does an excess of total energy for the organism as a whole bring about synthesis of fat from carbohydrate?

Body or depot fat may be synthesized from carbohydrates by reduction of the latter and liberation of oxygen; a speculative equation for this process follows:



From this equation it will be observed that from 100 gm. of glucose about 48 grams of tristearin and 47 grams of oxygen will result. Since this oxygen is endogenous, less oxygen from the air is required for respiration when fat is being synthesized by such a process, resulting in an apparent rise in the respiratory quotient. Under ordinary conditions in normal human beings the effect on the R. Q. would be slight, but in certain animals and fowls whose metabolisms are inherently fat-forming, such

as hogs and geese, if ample food is given, fat synthesis from carbohydrate causes a decided rise in R. Q. Bleibtreu (19), for example, showed that geese during luxus feeding gave respiratory quotients as high as 1.33. On starving these fattened fowls, this fell to as low as 0.72, showing that fat was being burned almost exclusively. Pembrey (20) found that marmots before hibernation eat excessive amounts of carbohydrates which are converted into and stored as fat and used during the hibernation period.

The chemical composition of stored fat is dependent on fatty acids of both endogenous and exogenous origin. Regarding the latter numerous investigations have shown that almost any of the higher fatty acids may pass into body fat, if they are eaten and absorbed at a time when fat is being stored. These include the acids which are known to be synthesized from carbohydrate, namely palmitic, oleic and stearic (and probably linolic) and in addition such common acids as myristic, linolic, linolenic, lauric, the highly unsaturated acids found in phospholipids and fish oils, arachidic and the like. In addition certain unusual fatty acids such as those which have been treated with bromine and iodine, erucic acid and chaulmoogric, when fed, are absorbed and deposited. In the case of chaulmoogric acid, the depot fat is optically active (11).

Many of these unusual acids have likewise been shown to pass into milk fat; hence they may be found in the butter fat of animals which have eaten them.

Investigations during the past few years by Eckstein (21), Powell (22) and Davis (23), have brought out the fact that certain of the acids of lower molecular weight, from C_4—C_{10} inclusive, do not appear in the depot fat; they are either completely oxidized or serve to synthesize higher fatty acids.

THE ESSENTIAL NATURE OF FATTY ACIDS.

Until quite recently fats have not been considered to be an essential dietary constituent. In (1929), however, Burr and Burr (24) described certain abnormalities in rats fed on a diet practically free from fatty acids, and believed to supply all other known dietary essentials. One of the outstanding manifestations of this condition was a dermatitis, especially noticeable on the tails. Linolic acid was found to be curative. While certain other investigators have disputed these results, they are especially interesting since they open up an entirely

new field of nutritional investigation. The future may disclose other essential functions of certain fatty acids.

On account of the limitations of space a more detailed discussion of the various factors related to fat metabolism has been impossible. There are obviously many gaps in our information on this subject. Only facts that are now generally recognized have been included. Future research on the fats and oils and especially study of that complex group called the phospholipids will not only give us a clearer picture of the details of fat transformations in cells but will disclose, no doubt, many new and important functions of these substances in living processes.

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Mosquitoes.

An addition to the taxonomic treatment of all the species of mosquitoes known to occur in N. A. A large amount of discussion is given on structures, biology, ecology, disease, transmission and methods of control. It is especially valuable for those interested in fresh water biology, public health work or medical entomology and can be used extensively in local areas for the study of mosquito problems.—D. M. DeLong.

A Handbook of the Mosquitoes of North America, by Robert Matheson. xviii+274 pp. Springfield, Charles C. Thomas. 1933.

THE RÔLE OF PROTEINS IN METABOLISM

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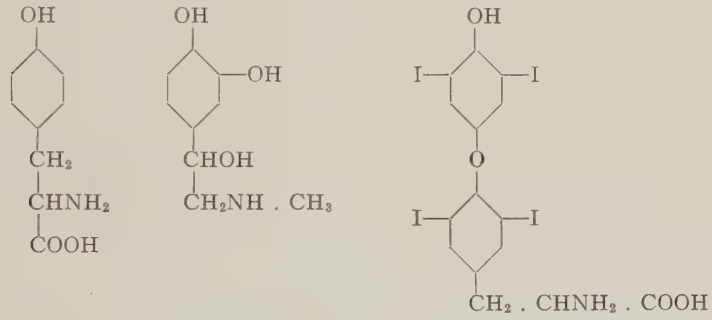
Proteins occupy a unique position in metabolism. Not only may they serve as sources of energy for the organism, but they constitute the most important raw materials out of which the complex structures of the body are built. While carbohydrates and fats are employed chiefly as fuels for the "protoplasmic fires," proteins, in the well balanced diet, serve mainly for purposes of synthesis.

Modern investigations have revealed the fact that proteins are composed of at least twenty amino acids. The amounts and methods of union of these acids vary widely in proteins of different sources. Indeed, no two proteins are exactly alike. Inasmuch as body proteins have their origin in those of the food, the latter must undergo complete disintegration before they can be utilized for synthetic purposes. This preparatory process is accomplished in the alimentary tract. At the completion of digestion the free amino acids pass unchanged into the portal circulation. Thence they are distributed throughout the organism, and serve as the substrates for the many synthetic transformations involved in the production of tissue constituents.

Very little information is available concerning the mechanism of protein anabolism. Each tissue protein is believed to be synthesized in the locality in which it is to exist. But the dominant factors which enable the cells to combine the amino acids with each other in the correct order, and in exactly the right proportions, are still beyond the realm of our comprehension. It may be shown mathematically that the number of possible isomeric proteins containing a single molecule of each of the twenty amino acids, united by the so-called peptid linkage, amounts to the incredible figure of 60×10^{20}. When one recalls that proteins are not made up of *single* molecules of the individual amino acids, but contain *many* molecules of each kind; and that several methods of union, instead of one,

may be employed in their synthesis, the number of possibilities is seen to be infinite. Thus the unerring accuracy with which specific proteins are synthesized out of the varying mixture of amino acids circulating in the blood is one of the most astounding attributes of living things.

But proteins are not the sole tissue components which originate in amino acids. Numerous non-protein nitrogenous materials arise in a like fashion. Thus, many of the hormones, and certain of the so-called "extractives" are manufactured from amino acids. Of the hormones, epinephrine and thyroxine are the best known chemically. Their structures indicate that they are probably derived from tyrosine or phenylalanine.

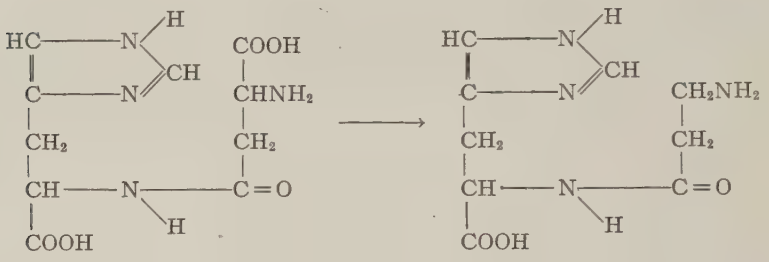


TYROSINE EPINEPHRINE THYROXINE

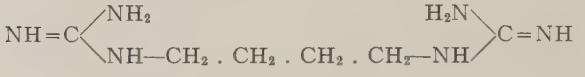
The mechanism of their formation, however, is still quite unknown. Diiodotyrosine, also found in the thyroid gland, may be an intermediate in thyroxine synthesis. The pancreatic hormone, insulin, appears to be a simple protein or polypeptid, and as such must be formed from the amino acids of the blood. Indeed, several amino acids have been recovered from the hydrolysate of crystalline insulin, notably, cystine, histidine, lysine, and tyrosine (du Vigneaud, Jensen, and Wintersteiner, 1927–28). Probably other internal secretions, such as those of the parathyroids, pituitary, and sex glands are derived from amino acids.

Of the extractives, carnosine, and its homologue anserine, isolated from muscle tissue by Ackermann and his associates (1929), are closely related to well known amino acids. The suggestion has been made that carnosine may be formed by the decarboxylation of the dipeptid, succinylhistidine. The recently described extractive, arcaine, discovered by Kutscher,

Ackermann and Flössner (1931) in the muscles of the invertebrate, *Arco noae*, may have its origin in arginine. Investigations of Ackroyd and Hopkins (1916), as well as those in the author's laboratory (Rose and Cook, 1925), appear to indicate that the purines, which are so important as components of the cell nuclei, are formed, at least in part, from the histidine of the diet.



Succinylhistidine. Carnosine.



Arcaine.

The extractive which has attracted the greatest interest, and occasioned the most controversy is creatine. This compound is the most abundant of the non-protein nitrogenous constituents of vertebrate muscles. It has acquired new importance in recent years because of its relation to muscular activitiy. The discovery of Fiske and Subbarow (1929) that creatine in the cells, exists for the most part in combination with phosphoric acid, opened the way for the elucidation of the extraordinarily complex process of muscular contraction. While all of the details are not yet clear, and are beyond the scope of this review, apparently the first change in the muscle, and the one which is primarily responsible for the contraction, is the hydrolysis of phosphocreatine into its components. This involves the liberation of about 11,000 calories of energy per gram-molecule of phosphocreatine decomposed (*cf.* Meyerhof and Lohmann, 1928). During the recovery or relaxation phase of muscular activity, the creatine and phosphoric acid are recombined at the expense of energy set free in the decomposition of carbohydrates or fats. The process is much more

complicated than our brief outline suggests, but the above will suffice to indicate the important role played by creatine in human economy.

The recognition of the function of creatine has served to renew interest in its origin. For many years attention was directed toward arginine as its most probable precursor. Doubtless this idea arose because arginine is the only known protein component which contains the guanidine group characteristic of creatine. Space does not permit a detailed review of the many efforts to augment the creatine content of the muscles, or the creatinine output in the excreta by the administration of excessive does of arginine. The majority of such investigations have yielded negative results. Even the prolonged (6–8 weeks) feeding to human subjects of daily doses of the amino acid equivalent to 1 gram of creatine failed to provide any evidence for a transformation of exogenous arginine into creatine or creatinine (Hyde and Rose, 1929).

In recent years attempts have been made to correlate creatine with other amino acids. Brand and his associates (1929–32) report that the administration of glycine to patients with progressive pseudohypertrophic muscular dystrophy may induce a 40 per cent increase in creatine excretion as compared with the control level without glycine administration. Furthermore, gelatin, a protein rich in glycine, is said to greatly enlarge the creatine output; while edestin, relatively low in glycine, exerts little effect. These observations have been confirmed and extended by Thomas, Milhorat and Techner (1932), who report a remarkable improvement in the clinical symptoms of dystrophy patients following glycine feeding. The sensation of fatigue so characteristic of the disease is said to diminish, and the function of certain muscle groups improves to the extent that activities formerly impossible can now be performed. The authors conclude "that glycine has an important function in muscle physiology, and a significant role in the pathogenesis and treatment of this disease."

Unfortunately, not all investigators are in accord with the view that creatine excretion is stimulated by glycine ingestion. Thus Abderhalden and Buadze (1932) report that the oral administration of 15 grams of glycine is without influence upon the creatine-creatinine metabolism of the dog. On the other hand, they present data indicating that histidine and purines are the precursors of creatine. They believe that the

relationship is a direct one in which the imidazole group is transformed into the extractive.

It is impossible at the present time to harmonize the results of the glycine and histidine investigations. One should note, however, that the conditions employed in the two studies were radically different. In the glycine experiments, diseased human patients served as the subjects, while in the histidine investigations presumably normal animals were used. In human dystrophy cases there exists unquestionably a creatine deficiency in the muscles, which doubtless is an important factor in the loss of muscular strength. Such individuals would appear to be particularly well suited for studies of the origin of creatine. But even after taking these facts into account no satisfactory explanation for the contradictory findings is evident. At this time we are interested primarily in emphasizing the extraordinary role which the amino acids play, both in the chemical make-up and in the physiological funtions of living things. Their uses in the normal individual, as well as their relation to the etiology of disease, are problems the solution of which will demand the greatest ingenuity of the investigator.

The multitude of synthetic reactions in which the amino acids participate cannot but excite one's curiosity as to the quantity of protein which is required for normal human nutrition. The controversy over the problem of the optimal protein intake, which was so actively debated for many years, is familiar to all students of metabolism. On the one hand, the Voit "standard" of 118 grams of protein per day, arrived at by a statistical study of a large number of human diets, was advocated with vigor by its proponents. They pointed out in no uncertain terms the dire consequences which would befall those who had the temerity to lower the protein intake "in the light of the accumulated evidence of the ill-effects that follow in the train of chronic underfeeding." At the other extreme, the disciples of the low protein regime were equally out-spoken in their denunciation of the evils of a high protein ration. One of these investigators expressed himself as follows: "The combustion of proteid within the organism yields a solid ash which must be raked down by the liver and thrown out by the kidneys. Now when this task gets to be over-laborious, the laborers are likely to go on strike. The grate, then, is not properly raked; clinkers form, and slowly the smothered

fire glows dull and dies" (Curtis, quoted by Chittenden, 1907, p. 269). Another exclaimed: "I have already said that it would seem to be practically impossible to avoid getting protein enough! Does it not appear to be quite in the order of things that, practically, we must always get enough protein for our needs if we eat as Nature dictates? How, otherwise, could mankind have progressed so well since some hundred thousand years before Christ until A. D. 1866, in which year Voit stepped on the stage with his standard? Nature makes such careful provision in all things that there could be nothing more certain than that a means of nourishment so indispensable to all animal organisms as protein actually is must be present in such generous measure in all our food-stuffs that there is not the slightest necessity to fear that we should be unable to get an adequate supply" (Hindhede, quoted by Mendel, 1923).

In the meantime, Chittenden, in extensive experiments upon himself, his students, and others, had demonstrated that nitrogen equilibrium can be secured and maintained with a daily intake of 45 to 55 grams of protein, a quantity less than one-half that of the Voit standard. The importance of Chittenden's observations, in showing the extent to which the protein content of the diet may be reduced under suitable experimental conditions, should not be underestimated. The determination of the minimal nitrogen intake compatible with the maintenance of the *status quo* of the cells was of fundamental significance. On the other hand, it does not follow that a very low intake is, under all circumstances, necessarily the optimal. When Chittenden's studies were first undertaken in 1902 much less was known concerning the chemical composition of proteins than is recognized today. The majority of the amino acids had been discovered prior to 1900, but very limited data existed concerning their relative distribution. Consequently, there prevailed little appreciation of the fact that the nutritive value of a protein depends just as truly upon the *kind* as upon the *quantities* of its components. Under the circumstances, it is not surprising that perhaps undue emphasis was placed upon the *amount* of protein ingested, and that scant consideration was given to possible differences in nutritive *quality*. Indeed, not until the advent of the Kossel and Kutscher (1900–01) procedure for the isolation of the diamino acids, and the Fischer (1901) ester method for the separation of the monoamino acids, were reasonably adequate

tools in the hands of the biochemist for the study of the make-up of proteins. The discovery of these valuable improvements in technic marked the beginning of a new era in our knowledge, not only of the chemistry of proteins, but indirectly of their physiological value as well. It was soon seen that proteins of different sources vary enormously as regards the proportions in which their constituent amino acids occur. Frequently, one or more amino acid was found to be missing entirely. Thus gliadin of wheat proved to be deficient in lysine; zein of corn was shown to be practically devoid of lysine and tryptophane; and gelatin was seen to be lacking in tryptophane, tyrosine, cystine, valine, isoleucine, and hydroxyglutamic acid (Dakin, 1920).

The recognition of these facts naturally raised the question as to the nutritive importance of the individual amino acids. Inasmuch as all of the generally recognized amino acids occur as components of tissue proteins, obviously each must be made available, either preformed in the diet, or by synthesis in the organism from other materials. Thus protein metabolism immediately became a much more complex phenomenon than was originally supposed. Instead of being concerned with a single dietary factor, it was now seen to involve each of the so-called "Bausteine" of proteins of which eighteen had been discovered by 1912. As stated by Osborne and Mendel in 1914, "Obviously the relative values of the different proteins in nutrition are based upon their content of those special amino-acids which cannot be synthesized in the animal body and which are indispensable for certain distinct, as yet not clearly defined processes which we express as maintenance or repair." (Osborne and Mendel, 1914a). As a result of this new view-point attention was directed in several laboratories toward determining which amino acids are necessary dietary components.

Among the earlier studies of the role of the individual amino acids in nutrition those of Osborne and Mendel are of extraordinary importance. These investigators (1914a) demonstrated the indispensable nature of tryptophane and lysine by feeding diets containing zein as the sole protein. They observed that young rats upon such rations not only fail to grow but rapidly lose weight. The addition of tryptophane to the food leads to maintenance but no growth, but the inclusion of both tryptophane and lysine is followed by rapid growth. In like

manner, when gliadin of wheat serves as the sole protein of the diet growth does not occur until lysine is incorporated in the ration (Osborne and Mendel, 1914a). Unquestionably, both lysine and tryptophane are indispensable dietary components. Furthermore, it is not necessary to supply the missing amino acids in the free state. The supplementation of a zein diet with some other protein containing adequate quantities of lysine and tryptophane results immediately in growth (Osborne and Mendel, 1914b).

In a similar fashion Osborne and Mendel (1915) showed that cystine is essential. When an otherwise adequate ration carries 18 per cent of casein, young rats receiving such a food mixture grow at normal rates. When, however, the proportion of casein is progressively diminished cystine becomes the limiting factor. At a 9 per cent level, casein is incapable of inducing normal growth; but the addition of cystine renders the diet adequate, and growth promptly ensues. Confirmatory evidence for the indispensable nature of cystine has been reported from several laboratories. Johns and Finks (1920) found that the addition of cystine to diets containing phaseolin markedly improves the nutritive quality of the food. Like results were secured by Sherman and Merrill (1925) in the use of a diet of whole milk powder overdiluted with starch.

Recently, Jackson and Block (1931, 1932) have made the remarkable observation that methionine may replace at least a large part of the cystine of the diet. These results have been confirmed by Weichselbaum, Weichselbaum, and Stewart (1932). It is not yet clear whether the substitution is due to a direct transformation of methionine into cystine, or to the possibility that each of the two amino acids may perform independently certain functions in which both ordinarily participate. Nor is it yet known whether a reverse replacement of methionine by cystine can occur.

A fourth indispensable amino acid is histidine. In 1916, Ackroyd and Hopkins observed that when arginine and histidine are removed from acid-hydrolyzed casein, the resulting material is inadequate for maintenance or growth. The authors state that if either arginine or histidine is included in the ration, no loss in weight occurs, and growth may be resumed. From these results they concluded that the two amino acids are interchangeable in metabolism, but that at least one must be present in the diet. In so far as the indispensable nature of

histidine is concerned, the experiments in the writer's laboratory completely confirmed the findings of Ackroyd and Hopkins (*cf.* Rose and Cox, 1924). We were unable, however, to demonstrate an interchangeable relationship between the two amino acids. The addition of histidine to the diet invariably induced an immediate and rapid increase in weight of the animals, but the inclusion of arginine exerted no influence upon growth even when the quantity added was more than equivalent to the sum of the arginine and histidine present in casein. Confirmatory evidence in support of the essential nature of histidine was furnished by later publications from the writer's laboratory (Cox and Rose, 1926), and by Harrow and Sherwin (1926).

Thus, these four amino acids—tryptophane, lysine, cystine, and histidine—are known to be absolutely indispensable dietary components. In the absence of either nutrition fails, and eventually death results regardless of how much other food is consumed. The probability exists that either tyrosine or phenylalanine is also a required dietary component. Recently, at the University of Illinois, we have undertaken a study of the effect of combined crystallization and selective absorption as a method of removing amino acids from hydrolyzed proteins. While the investigation is not yet completed, a material has been obtained from casein which when suitably supplemented with certain purified amino acids, including tyrosine and phenylalanine, supports satisfactory growth. In the absence of tyrosine and phenylalanine, growth is impeded. At an early date we shall know whether one or both of the compounds in question must be present in the food. We are confident that at least one is indispensable. Several years ago Abderhalden (1915, 1922) presented data which he interpreted as indicating that either tyrosine or phenylalanine must be included in the food, but that the two are mutually interchangeable in metabolism. On the contrary, Totani (1916), and Lightbody and Kenyon (1928) were unable to demonstrate any relationship between the growth of rats and the tyrosine content of the diet. The conflicting results in the literature necessitated the investigations in which we are now engaged. So far our findings confirm those of Abderhalden.

In contrast to the amino acids discussed above, much evidence is available indicating that certain protein components may not be necessary. It is well known that when

benzoic acid is administered to man or to most animals it is conjugated with glycine, and is eliminated in the urine as hippuric acid. By measuring the maximum production of hippuric acid several investigators have reported that the output may carry more glycine than is found preformed in the proteins metabolized. The origin of the glycine is unknown, but it has been shown that a considerable portion of the nitrogen which in the normal metabolic processes is converted into urea, may, after excessive doses of benzoic acid, be diverted to the synthesis of hippuric acid. These findings led to the general impression that glycine may be synthesized by the organism out of ammonia and non-nitrogenous materials, or from other amino acids. As further evidence in this direction the fact has sometimes been emphasized that casein, though low in glycine, serves admirably for purposes of growth in both man and animals (Abderhalden, 1915). In like manner gliadin and zein, both of which are believed to be devoid of glycine, are made satisfactory for growth by suitable supplementation without the addition of glycine.

The evidence, however, is not all in favor of the dietary dispensability of this amino acid. Griffith (1929–30) has shown that the growth of young rats may be inhibited by the inclusion of benzoate in the diet unless glycine as such, or in the form of protein, is supplied in amounts sufficient to detoxicate the benzoate, and meet the needs of tissue synthesis. While the author believes that his data "support the idea that glycine is synthesized by animal tissues," evidently the synthesis is limited in extent. Apparently, the use of glycine for detoxication purposes may create a deficiency for the growth function unless an increased supply of the amino acid is provided. In the light of these investigations one must conclude that the prevailing idea that glycine may be formed practically *ad libitum* by the animal organism is at the present time scarcely warranted.

The relation of arginine to maintenance and growth has been the subject of several investigations. Reference has already been made to the papers of Ackroyd and Hopkins and of Rose and Cox involving the feeding of casein digests from which both arginine and histidine had been precipitated. According to Abderhalden (1922) arginine is probably indispensable in nutrition. His investigation, involving the use of mixtures of purified amino acids, is in some respects remark-

able; but owing to the difficulties experienced in the synthesis of the dietary components, the available materials were necessarily limited, and the feeding trials were few in number and of short duration. His results appear to be open to the further criticism that frequently his animals were provided with inadequate supplies of vitamins. Using an entirely different procedure, Crowdle and Sherwin (1923) report that fowls are capable of synthesizing ornithine for the detoxication of benzoic acid. Since ornithine is a component of arginine, the observation suggests that the latter also may be a synthetic product, at least in the species in question.

In view of the uncertainties inherent in the above experiments the arginine problem was attacked by a different method (Scull and Rose, 1930). This involved a comparison of the arginine intake of growing rats on an arginine-low diet and the increments in tissue arginine, in order to determine whether the latter may be accounted for by the amounts of the amino acid in the basal rations and vitamin supplement. For this purpose, hydrolyzed casein was rendered as nearly devoid of arginine as possible, and was incorporated in a diet which was administered *ad libitum*. At the beginning of the experiments, litter mates of the animals employed in the growth studies were killed and subjected to analysis *in toto* for arginine. The other members of each litter were killed and analyzed after they had received the experimental diet for a period of 64 days. In the meantime they had gained 73 to 113 grams each. Without exception the increase in tissue arginine was 2 to 3 times as large as could be accounted for by the total arginine content of the food. The findings "seem to warrant the conclusion that arginine may be synthesized by the organism of the rat, and in this species at least is not an indispensable dietary component" (Scull and Rose, 1930).

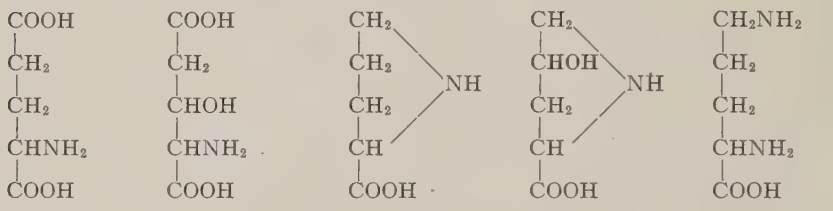
Investigations concerning the relation of certain other amino acids to nutrition have been made, but at the present time the results scarcely warrant positive conclusions. A number of years ago Abderhalden (1912) suggested that proline might be a dispensable amino acid. On the other hand, Sure (1924) is of the opinion that it is necessary. His data, however, are not very convincing. St. Julian and Rose (1932) have removed proline as completely as possible from hydrolyzed proteins by 40 extractions with hot absolute alcohol, without impairing the growth-promoting value of the resulting material. The

same investigators have precipitated the dicarboxylic amino acids, glutamic, hydroxyglutamic, and aspartic acids, without diminishing the nutritive properties of the residue. Hopkins (1916) is of the opinion that neither glutamic nor aspartic acid is indispensable. Sherwin, Wolf, and Wolf (1919) report that in the human subject glutamine may be synthesized for the purpose of detoxicating phenylacetic acid, which in man is excreted in the urine as phenacetyglutamine. Hydroxyglumatic acid is usually listed as non-essential inasmuch as edestin, which presumably is devoid of this amino acid, supports normal growth (*cf.* Osborne and Mendel, 1915; and Osborne, Leavenworth, and Nolan, 1924).

Very little information is available concerning the possible nutritive importance of hydroxyproline. Spörer and Kapfhammer (1930) call attention to the fact that several vegetable products, notably soy-bean flour, do not contain detectable amounts of this amino acid. The authors suggest that in view of the quantitative importance of soy-beans in the dietaries of oriental peoples, hydroxyproline may not be an essential constituent. More recently, Adeline (1931) has reported that rats upon diets containing 6 per cent of edestin cease growing after approximately 12 weeks, but that the addition of either proline or hydroxyproline remedies the nutritive deficiency, and induces growth. She concludes that the two compounds are interchangeable in metabolism. Unfortunately, the data are not so extensive or clear-cut as one would wish, in view of the conflicting evidence regarding the subject.

The interpretation of the results of feeding experiments in which proline, hydroxyproline, and the dicarboxylic acids are supposedly absent from the diet is rendered difficult by the lack of delicate tests for these amino acids. In such investigations one must recall that even traces of a life-essential may suffice to meet the growth requirements of the organism. It is possible that the prolines, glutamic acids, and the ornithine part of arginine may all be interchangeable in metabolism, or be capable of yielding a single essential. Thus if proline were necessary but absent from the food, one or more of the other four might be transformed into the missing amino acid, and thus prevent a dietary deficiency. The similarity in structure of the five compounds is readily seen from the accompanying formulas. Indeed, Abderhalden (1912, 1922) has frequently suggested that glutamic acid and proline may be

capable of replacing each other. We have attempted to secure evidence for such a substitution by removing all five amino acids from hydrolyzed casein (St. Julian and Rose, 1932). The resulting material was supplemented with cystine, tryptophane, and histidine, and was incorporated in the diet at a level of 11.5 per cent (including the supplements). The rats which received this ration each gained at a rate of approximately 1 gram per day. The addition to the food of arginine, glutamic acid, aspartic acid, and proline failed to accelerate the increase in body weight. In view of the low level at which the hydrolysate was fed, it is difficult to interpret the results on any basis other than that the amino acids in question are not necessary dietary components.



GLUTAMIC ACID — HYDROXYGLUTAMIC ACID — PROLINE — HYDROXYPROLINE — ORNITHINE

Very recently, Wada (1930, 1933) has reported the isolation of a new amino acid, citrulline, from a tryptic digest of casein. The compound is said to have the following formula:

$$O = C\begin{matrix}NH_2 \\ NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH\end{matrix}$$

As will be observed, it is closely related to arginine. However, its presence in casein does not invalidate the experiments of St. Julian and Rose inasmuch as during acid hydrolysis citrulline is transformed into proline, and in this form is removed by repeated alcohol extractions. Final solution of the problem of the relation of the prolines, glutamic acids, arginine, and citrulline must await the use of a diet containing a synthetic mixture of amino acids known to be entirely devoid of the materials in question.

The present status of knowledge regarding the relation of the amino acids to nutrition is summarized in Table I. Of the twenty generally recognized protein components, the indispensable nature of only five has been positively established.

Two others, methionine and phenylalanine, appear to be capable of substituting for cystine and tyrosine respectively. The importance of seven is at the present time uncertain. Concerning the remaining six, available information does not warrant their classification with respect to maintenance and growth.

The bearing of the above facts upon the problem of the optimal protein intake is evident. In view of the well-known deficiencies of many proteins, notably those of plant origin, it would seem to be a safer procedure to consume more than the minimal amount necessary to maintain nitrogen equilibrium.

TABLE I.

TENTATIVE CLASSIFICATION OF AMINO ACIDS WITH RESPECT TO THEIR NUTRITIVE IMPORTANCE.

Indispensible Amino Acids	Amino Acids which have not been definitely placed, but which appear to be dispensable	Amino Acids of unknown nutritive importance
Lysine	Glycine	Alanine
Tryptophane	Arginine	Serine
Cystine (or methionine?)	Proline	Valine
Histidine	Hydroxyproline	Leucine
Tyrosine (or phenylalanine?)	Aspartic acid	Isoleucine
	Glutamic acid	Norleucine
	Hydroxyglutamic acid	

Perhaps a suitable daily intake of protein for an adult of average size might be placed at 70 to 75 grams, or about 1 gram per kilogram of body weight per day. Such a quantity allows approximately 50 per cent surplus, as compared with the average Chittenden standard, in order to insure the presence of the essential amino acids in sufficient amounts.

The fundamental nature of investigations regarding the nutritive role of the individual amino acids need scarcely be emphasized. Obviously, adequate interpretation of the facts of protein metabolism, especially with respect to the growth process, will remain impossible until the importance of each amino acid has been determined. It appears that if further information of this sort is to be secured, one must either devise more adequate methods for the quantitative removal of single components of proteins, or resort to the use of synthetic mixtures

of highly purified amino acids. The latter alternative seemed to us to be the more promising, and has already yielded results of considerable interest.

Several attempts have been made in the past to feed mixtures of purified amino acids in place of proteins, but hitherto all such efforts have met with failure. Animals upon such rations have invariably declined in weight and finally died. For about three years experiments of this sort have been under way in the author's laboratory. In formulating the amino acid mixture we imitated the composition of casein in so far as available information permitted. Nineteen amino acids were used. Hydroxyglutamic acid was the only recognized protein component not incorporated in the food. That its absence was not significant was shown by supplementing the ration with a crude fraction of protein containing the dicarboxylic acids. The rats receiving the diets rapidly lost weight during the first 12 days, and then declined gradually or maintained weight to the end of the experiments (Rose, 1931). The results were interpreted as indicating that growth-promoting proteins contain at least one essential component other than the twenty known amino acids. In line with this conclusion, it was found that the addition to the diet of 5 per cent of casein, gliadin, or gelatin in place of an equivalent quantity of the amino acid mixture, was followed after 4 days by slow growth (Ellis and Rose, 1931). Evidently the supplements furnished something which was lacking in the amino acid mixture.

Inasmuch as casein proved to be more effective in stimulating growth than did either gliadin or gelatin, the former was employed in attempting to concentrate the active material (Windus, Catherwood, and Rose, 1931). For this purpose, hydrolyzed casein was fractionated into five groups of amino acids, namely, the less soluble ones, the dicarboxylic acids, the diamino acids, the alcohol-soluble material (proline), and the monoamino acids. The first four groups proved to be almost or completely devoid of growth-stimulating activity. On the other hand, the fraction of monoamino acids carried the unknown essential in much greater proportions than does whole casein. Indeed, by a second fractionation of part of the monoamino acids a material was obtained which induced normal growth when present in the food to the extent of 5 per cent.

The compound has been concentrated by several other methods of protein fractionation. By the use of the Town (1928) copper salts procedure, the growth essential is found to be associated with those amino acids whose copper salts are soluble both in water and in anhydrous methyl alcohol (Caldwell and Rose, unpublished data). In our best preparations, the chief contaminants of the unknown substance are believed to be valine and isoleucine. Incidentally, the compound is not identical with aminobutyric acid (Foreman, 1913; Abderhalden and Weil, 1913), the amino acids described by Schryver and his associates (1925–1927), norvaline (Abderhalden and Bahn, 1930), or any of the other protein disintegration products which have been mentioned from time to time in the literature. It appears, therefore, not to have been recognized heretofore.

Already we have learned many of its properties. These are being made use of in its further concentration. We are also conducting a study of its distribution in other proteins. We hope eventually to accomplish its isolation and identification. If we succeed, as we anticipate, we shall then be in position to determine with comparative ease which of the remaining amino acids are required for normal nutrition.

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A Case of Sex-Reversal in Man.

This curious story is the account of a well-known Danish painter, Einar Wegener, who started out in life as an outwardly normal male. He married, and lived an apparently normal life for some years. Gradually, however, he felt himself becoming psychologically a woman. No homosexual tendencies were evident. This psychological change continued to the point where, after being ridiculed by many physicians, he was at last taken in hand by an eminent Dresden specialist, who removed his sex organs, found rudimentary ovaries, implanted fresh ovaries, and made of the artist an essentially normal woman. The account is said to be strictly authentic and accurate. It is written in popular style, with no attempt at scientific analysis. A foreword by Dr. Norman Haire of London is more scientific, and verifies the essential facts of the book. Certain incidents strain the imagination somewhat, such as the change in the character of the handwriting from that of a man to that of a woman immediately following the first operation. Biologists and psychologists will find this unique story of considerable interest, as there would appear to be much debatable ground as to the interpretation of the initial changes in Wegener's makeup.—L. H. S.

Man into Woman. Edited by Niels Hoyer. 288 pp., 18 ill. New York, E. P. Dutton & Co., 1933. $3.50.

CRITICAL SITUATIONS IN THE MINERAL METABOLISM OF HUMAN BEINGS AND DOMESTIC ANIMALS

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As I think of animal life, metabolism is one indivisible system—mineral metabolism existing only as we choose to concentrate our attention on the mineral elements which are involved in all metabolism; but, for convenience sake, I concede the conventional point-of-view, and have chosen to discuss those conditions relating to mineral metabolism of man and domestic animals which frequently intrude themselves upon our attention.

Since I happen to have grown up in the family of a zoologist it is my habit to think of the nutritive requirements of man and his animals as having been determined primarily during the evolution of the species, and only secondarily as modified since the dawn of civilization.

The fundamental situation with which we have to do, therefore, is the natural harmony between the animal and its environment—the adjustment between metabolism, structure, nutritive requirements and food supply—which arose by processes of evolution, and was maintained by natural selection, before the development of the human mind brought a new and profoundly disturbing element into the situation.

Incidentally, this was only yesterday, in the history of the species.

What, then, has happened—what has mankind done—to disturb this ancient harmony, and to bring upon us critical situations with reference to mineral nutrition?

These disharmonies in the nutritional affairs of human beings began with the use of fire in the preparation of food—with resulting injury to the vitamins, which, either directly or indirectly, influence mineral metabolism. This applies, especially, to vitamins A and C, which are readily damaged by heat and oxygen; vitamin B, which is sensitive to heat and moisture, and vitamin G, which is destroyed by dry heat.

Also cooking renders food pasty, so that it sticks to the teeth, and undergoes acid fermentation.

Furthermore, the cooking of food greatly diminishes the need for use of the teeth; and thus tends to diminish the circulation of blood to the jaws and teeth, and to produce underdevelopment of the maxillary and contiguous bones—thus leading to contracted dental arches, and to malocclusion and impaction of the teeth, with complications of great seriousness.

The second outstanding incident among the nutritional adventures of mankind was his adoption of agriculture as a method of living—especially because it led to a diet based upon cereal seeds and seed products, with large use of sugar. In the manner in which we use cereal foods they tend toward deficiency of virtually all nutrients that are ever lacking in the human diet except energy-producing nutriment, and vitamin E—which controls reproduction.

A third incident of significance in relation to mineral metabolism of human beings—the taking up of residence in houses, with roofs over them, and with glass windows, has added further to one of our nutritional difficulties by diminishing our contact with the short, chemically potent, ultra-violet light waves, which transform the ergosterol of the skin into vitamin D—thus endowing it with power to catalyze the most efficient utilization of calcium and phosphorus.

It is true that agriculture has also increased the available supplies of those classes of foods which are nutritionally better balanced than are refined cereal products—namely, meat, milk, fruits and vegetables—but out of the situation as a whole has come a new freedom to use nutritively deficient foods, of which we have availed ourselves, and an obligation to balance our diet, by the use of other kinds of foods, which we have not had the wisdom and the will to satisfy.

The fault is not with agriculture, but with humanity.

Manufacture, transportation, and commerce have also contributed to this new freedom and new responsibility in food selection; soil exhaustion has served to diminish the content—in some foodstuffs—of some mineral nutrients, especially iodine; and cultivated tastes dictate that we throw away valuable mineral nutrients in the skins of vegetables, in the skins and cores of fruits, in the less palatable parts of cereals, and in discarded cooking water.

Coincident with these changed conditions of diet and habitation have arisen certain minor changes in mankind himself—through diminished severity of natural selection, and

the substitution therefor of selection on bases of indifferent or of definitely disgenic effect.

This cessation or diminished severity of natural selection applies in a general way, as life has become easier, but is especially significant in relation to mineral metabolism in connection with the teeth, with fat digestion by infants, and with the function of lactation.

Thus, before the days of cooking and milling, the teeth of mankind had a definite survival value which they do not possess today, and so were maintained by natural selection at a certain level of excellence and efficiency.

The most difficult constituent of milk for an infant to digest is fat. If fat is not digested it may unite with alkali mineral elements in the alimentary tract, the products being carried out of the body in the chemical form of soaps, thus creating, in some cases, conditions of alkali deficiency and relative acidosis.

As we read the names of the many children in the families of our early American ancestors it is shocking to note the large percentage who died in infancy; but those pathetic sacrifices maintained the level of digestive competency on a higher plane than now prevails.

Similarly, the modern dairy industry has eliminated the survival value of the lactating function in womankind, and the capacity of the human mother to provide mineral as well as other nutriment to her infants has diminished. The cow helps us to "get by," and to hand on down nutritional problems of increasing difficulty.

That there has been an intensification of the severity of natural selection of human beings through a shift of emphasis from physical to certain mental and other nervous capacities, is indubitable. Thus the thousands of "jay-walkers" who awake, each year, in the land of the hereafter, must assist, willy-nilly, in the maintenance of our "sense of whereatness," but the net effect of the shift unquestionably leaves us with accentuated problems of mineral nutrition.

While, therefore, we need give no thought to the possibility of progressing backward to the nutritional situation of aboriginal man—living in natural harmony with his environment—we need at least to realize why we are where we are; which way we are progressing; and what it is practical to do about the nutritional disharmonies of the present.

CARIES.

Caries (1–5), or tooth decay of young people—an almost universal disorder of mineral metabolism among the civilized, and of immense importance in relation to arthritic, cardiac and renal disease—is at this time the subject of extensive investigation.

My view of this subject is merely that of the student of nutrition; but inasmuch as the dental pathologists are not in agreement, and have not solved the problem, we expect them to forgive us our unprofessional interest.

The most outstanding fact relating to caries is that it is primarily a disease of civilized mankind, in the sense that among many primitive peoples caries is remarkably less prevalent. Among possible contributory factors the following are considered by students of the subject, from various points of view:

1. Adhesive diets, especially such as contain much starch and sugar, yield acid, on fermentation, which destroy tooth enamel.

2. The impaction of carbohydrate foods, in situations leading to compression, contributes to fermentation effects.

3. In certain experimental work the size of food particles has seemed to be significant in relation to adhesion, impaction and fermentation.

4. Mucin plaques provide an environment favorable for decalcifying fermentation.

5. The quantities of calcium and phosphorus in the food, and the proportion of the quantity of the one to that of the other, as affecting the quantities and proportions of the same in the blood and the saliva, and especially as affecting the hydrogen-ion concentration and the acid-neutralizing capacity of the saliva, are believed by some to be significant.

6. Dietary deficiencies, in vitamins C, D and B, are said to be important in this relation; especially since deficiency of vitamin C may cause a separation of the odontoblastic layer from the dentine, and other associated abnormalities and degenerative changes; and since deficiency of vitamin D during the developmental period may cause the teeth, when erupted, to be defective in placement and structure.

7. Puberty and pregnancy are discussed as contributory causes.

8. Rickets, perhaps because of origin in part in the same conditions, is considered as predisposing to caries.

9. The presence of fluorine in foods or in drinking water is prejudicial to normality of dental structure.

But granting—for the sake of the argument—the validity of all these suggestions, I suspect that we have not yet mentioned the principal cause of caries, which appears, to my practical sense, to be disuse. The problem of caries, therefore, as I see it, is as to whether any combination of conditions as to nutrition and of environment will produce and will maintain normal tooth structure in the absence of the use to which teeth were adapted during the evolution of the species.

In this light, I doubt whether civilized mankind will ever have as good teeth as do some of the primitive peoples—unless we go back to diets which require vigorous chewing. When we chew by machinery we release the tension—physiologic and genetic as well—which, in a state of nature, maintains the teeth in a condition of normal excellence.

For the present, at least, therefore, I think that the practical question is as to the best compromise we can make with nature. To that end I would keep informed as to results of research in progress, and would attempt to prevent caries by dental treatment and dietary practice based especially upon the following:

1. The prevailing doctrine of the dental sanitarians—based upon the ideas of Miller, and those who have followed, and modified, and extended his conclusions as to the significance of the acid fermentation of carbohydrates;

2. The conclusions of Mrs. Mellanby as to the importance of vitamin D in relation to tooth development, in the prenatal as well as the postnatal stage;

3. The ideas of Bunting and associates, and many others, of the importance of a low-sugar diet, and an effective dental antiseptic.

4. The findings of Howe, as to the importance of vitamin C, and of Hanke and associates as to the efficacy of liberal use of citrous fruit juice;

5. The evidence, from many sources, as to adequate calcium and phosphorus contents of the diet; and, above all,

6. The observations of Waugh (6), and of others, on the teeth of primitive peoples, which seem to indicate the importance of vigorous use.

The Eskimos have the largest jaws and best teeth of any existing race, but after a single generation on the white man's diet, there is marked degeneration in the size and development of the jaws, and in the regularity and soundness of the teeth. Waugh concludes that "the American Eskimo is veritably paying for his civilization with his teeth."

MOTTLED ENAMEL.

Another abnormality of the teeth, the cause of which as a mineral disorder has only recently been discovered, is mottled enamel.

The enamel of the teeth becomes dull in appearance, chalky, pitted, structurally weak, lacking in cementing substance between the rods, and as a secondary effect may become variously stained.

Black and McKay (7), and H. V. Churchill (8) published early studies relating to this condition in the United States; but Margaret C. Smith (9), and associates, of the Arizona Agricultural Experiment Station, first clearly showed that the mottling of enamel is caused by fluorine in the drinking water, by producing the mottling by administering sodium fluoride.

Since this difficulty arises only from the use of drinking water from certain particular sources, the remedy is obvious.

ANEMIA.

Simple anemia is known to all as an iron-deficiency disease. The iron-poor foods of greatest importance in this relation are white flour, sugar and milk; and the foods of greatest importance because of their richness in iron are red meats and liver, green vegetables and eggs.

Anemia is common in infants (10)—whether naturally fed or bottle fed—and they respond readily to iron medication, which suggests that the aboriginal baby cut its teeth on a bone, and got some valuable shreds of meat at an early age.

The silly anti-meat propaganda of the recent years, seems about to have spent itself, and pediatric practice is swinging back toward the attitude of our parents and of our remote ancestors in the matter of meat for children.

Smythe and Miller (11) found that the percentage iron content of the body of the albino rat diminishes by a half during the suckling period, but rapidly increases to the normal

after solid food is taken, which throws light upon the situation of the human infant.

It is possible to get the supplementary iron required by infants from vegetable foods, and from drugs; but in my opinion beef juice, scraped beef and egg yolk are more natural sources of iron, and highly desirable dietary components on other accounts.

When I was a child our old-fashioned family doctor recommended steel saw filings, on bread and butter, for my sister's anemia; and the treatment was efficacious even beyond expectations, because my sister not only recovered from her anemia but became, and is today, a violinist—which testimonial for saw-filings should be noted by manufacturers of saws and files, as well as violins.

Miller and Forbes compared protein foods of many kinds as sources of iron, and found the meats to be the best and milk the poorest of those studied, in this respect.

Whipple and Mrs. Robscheit-Robbins (12) made an unusually extensive series of studies of the blood-building values of foods, with dogs as subjects. While it was clear that iron is important in this respect, it was equally clear that something else—found even in fruits—contributes to the ability of the dogs to synthesize hemoglobin.

Important later progress in this relation was the discovery of E. B. Hart and associates (13) that a trace of copper is effective as a supplement to iron for hemoglobin synthesis. Copper is contained in many, at least, but not in all foods.

Many vegetable foods contain approximately 2 parts of copper per 1000 of fresh substance—milk containing only one-third of this quantity—while some vegetables, and nuts, and cereals, contain several or many times this quantity. It is highest in the germs of seeds, which suggests its connection with the active metabolism of these parts (14).

Copper is not usually in deficient supply in otherwise practical diets, but may be so in particular cases.

Pernicious anemia is caused by an organic deficiency of the stomach, and is not within the field of this discussion of mineral metabolism.

THE IODINE PROBLEM.

One of the most important and clear-cut situations in the field of mineral nutrition is the relation of the iodine intake to the function of the thyroid, and to associated functions (15).

The thyroid gland regulates the rate at which the fundamental chemical reactions of the body are carried out, and is intimately related to the heat production.

Simple goiter is a hypertrophy of the thyroid, the first cause commonly being deficient intake of iodine.

Among the organs of the body, in addition to the thyroid, the hair, skin and nails are comparatively rich in iodine (16), and the condition of these parts is intimately related to the thyroid, and to iodine metabolism.

Seaweeds, burnt sponge, and preparations of the thyroid, which are exceedingly rich in iodine, had been used by the Chinese in the treatment of goiter for thousands of years before the discovery of iodine by Courtois in 1811.

In 1820 the chemist Dumas and the physician Coindet learned that iodine would cure some cases of goiter; but not until 1850 did Chatin advance the hypothesis that the deficiency of iodine in certain countries causes goiter; and two years later he determined that the iodine content of foods differs in accord with the iodine content of the soils on which they are grown. Recently it has been learned that the iodine content of milk and of eggs can be similarly modified, by iodine feeding of the animals which produce them.

In 1895 Baumann discovered iodine in the thyroid, and in 1914 Kendall isolated thyroxin, the active principle of the thyroid, containing 65 per cent of iodine.

Sea water contains iodine, as also do marine organisms. Deposits of salts formed by evaporation of sea water, therefore, contain iodine, but in the usual preparation of salt for nutritional or for technical use the iodine is lost. If the total iodine were retained there would be 2000 parts of iodine per billion of salt. It is much easier to restore iodine to refined salt than to retain it through the process of refinement.

The iodine content of drinking water varies enormously. McClendon and Williams, and McClendon and Hathaway found iodine in city water supplies to vary between 184.7 parts per billion in a deep well water from Mexia, Texas, and very small fractions of one part per billion in many cases. In the Scioto river water at Columbus, Ohio, they found 0.21 of one part per billion of iodine.

The iodine content of foods varies much in different parts of the United States, and the incidence of goiter likewise varies,

in general harmony with iodine deficiency of foods and drinking water.

Among the foods containing the highest quantities are oysters, clams, marine fish and Irish moss. Red cabbage is comparatively rich in iodine; whole potatoes and apples are much richer in iodine than are these foods after being peeled; and vegetables must lose a large part of their iodine into the water in which they are cooked; but immeasurably more important than the kind of food, in this relation, is the iodine content of the soil. Foodstuffs in general contain enough iodine, except as the soil on which they are grown is deficient.

In 1917 Marine and Kimball experimented in the schools of Akron, Ohio; 2190 school girls took 2 grams of sodium iodide twice a year for 3 years. Of these only 5 developed goiter, while of 2,305 girls not given iodine, and observed as controls, 495 developed goiter. The results of this experiment were so striking and convincing that iodine prophylaxis for goiter was taken up in many parts of the United States, New Zealand and India. The administration of iodine for the prevention of goiter is under government control in Switzerland and Italy.

Minimum effective iodine dosage has not been satisfactorily established. The use of iodine, and the quantity used, should be determined by a physician.

Eggenberger states that the biological minimum iodine intake to prevent goiter in human beings is 0.04 mg. per day—which would be a total of about 15 grams per year.

Hathaway advocates iodine surveys of all goitrous regions, the addition of sodium iodide to the water supplies of cities and towns, and the use of iodized table salt or tablets in rural communities.

The addition of sodium iodide to the city water supplies is practiced in several cities in this country, but it is cheaper for the people to use iodized salt, if they will do it, since only a small part of a city water supply is used for drinking.

O. P. Kimball (17) says that every iodine survey emphasizes the importance of goiter prevention during pregnancy. "We cannot stress this point too strongly and in endemic goiter districts we need the attention of the physicians who educate and direct women through this important period, and of every prospective mother. It is during pregnancy that the general

use of iodized salt will be of greatest value and should be used in every case unless otherwise directed by a physician."

"The history of this problem for the past century teaches most emphatically that the treatment of goiter is very unsatisfactory and accomplishes very little toward the control of the disease, but those attacking the problem from the viewpoint of preventive medicine—physiologists, biochemists and public health authorities—have accumulated sufficient evidence to justify the assertion that endemic goiter is the easiest known disease to prevent."

RICKETS.

Numerous surveys of infant nutrition have shown that affliction with a slight degree of rickets is exceedingly common throughout the temperate zone (18), and that a large part of these cases recover, as abundant sunshine and mixed diet replace the indoor confinement and the milk diet of early infancy, without this condition being recognized. In fact, the human infant seems to live close to the border line of rickets, because woman's milk and cow's milk as well contain, at the most, only a narrow margin of excess vitamin D, which is essential to the most efficient utilization of calcium and phosphorus.

Aside from the factors mentioned, refined cereal foods are most largely responsible for the existence of this disease, as they crowd out of the diet other foods containing more of the bone-growing requisites.

Man is one of the slowest-growing of all animals; he is adapted to and is provided with a comparatively low-calcium diet; and he is not capable of tolerating much interference with the utilization of his mineral nutrients. These facts imply that during the development of the species the conditions preventive of rickets—among which is sunshine—were dependably present in requisite quantities.

In spite of the much higher calcium and phosphorus content of cow's milk than of woman's milk the infant fed on cow's milk is much more prone to rickets than is the infant which receives its mother's milk—the calcium and phosphorus of cow's milk being not so well utilized.

The utilization of the calcium and phosphorus of milk by an infant, and therefore its tendency to develop rickets, is affected by many conditions, as to food supply and preparation, environment, digestive capacity, and rate of growth (19–24).

Some of these conditions are:

1. The quantities of calcium and phosphorus present in the diet.
2. The ratio of calcium to phosphorus in the diet.
3. The vitamin D content of the milk, and other constituents of the diet.
4. The exposure of the infant to solar radiation.
5. Rapidity of growth—which, if extreme, may contribute to the production of rickets.
6. Vigor of digestion—especially capacity to digest milk fat, which if not digested interferes with the utilization of calcium and phosphorus.
7. The time of year—as determining the intensity of the sunshine, and the vitamin values of foods.

The usual causes of rickets are combinations of unfavorable conditions as to these factors. It is normally curable by removal of the causes, and the provision of conditions sufficiently favorable for the nutrition of the bones.

Artificially fed infants, and many naturally fed infants as well, need supplementary vitamin D. It becomes a practical question, then, as to how this nutrient shall be provided—which may be in cod-liver oil, in other such oils, in concentrates prepared from such oils, in irradiated ergosterol, or in irradiated foods containing ergosterol—or it may be formed, in the skin, by exposure to sunshine or to special ultra-violet irradiation.

Irradiation by direct sunshine, or by mercury vapor or carbon arc lamps, is much more effective than is dependence on the penetration of special window glass by ultra-violet rays of sunlight.

Very many foods can be enriched in vitamin D by irradiation, and, so enriched, they are endowed with potency to heal rickets.

Milk can be enriched in vitamin D by feeding irradiated foods or drugs to the cow; but this is not a quantitatively efficient process—direct irradiation being much the most effective method of accomplishing the purpose.

The University of Wisconsin Alumni Research Foundation recently announced the perfection of an apparatus for the commercial irradiation of milk. The device consists of an upright cylinder down the inner walls of which the milk flows in a thin sheet, being exposed, during the process, to irradiation from a 12,000-watt battery of electric lamps which emit ultra-

violet light, the milk acquiring vitamin D potency about equal to that of good cod-liver oil.

It is possible to take too much vitamin D (25), with the result that calcium may be deposited in the blood vessels, heart, stomach, lungs, kidneys and muscles, and that there is an excess of calcium in the blood as well. Responsible guidance in this relation, therefore is necessary.

The prevention of rickets should begin with the care and feeding of the expectant mother (26). Her diet will affect the vitamin D content of her milk.

Among the foods deserving of mention as contributing to the rickets-preventive capacities of the child's diet, milk deserves first mention, because it is rich in calcium and phosphorus, but by itself cannot be regarded as a rickets-preventive, because it is not rich in vitamin D. Egg yolk is rich in vitamin D; butter is a fair source of D; while cod liver and other fish liver oils are very rich in this nutrient.

THE MINERAL NUTRITION OF LIVESTOCK.

The mineral nutrient problems of domesticated animals may be classified as of geographical or of physiological origin.

The deficiencies of geographical origin—which are dependent on soil and forage composition—have to do with phosphorus, iron and iodine especially, but also with calcium, and affect the different kinds of animals in accord with the proportion of forage in their respective rations, and with the magnitude of the demands for mineral nutriment which are made upon them by their different lives and functions.

HORSES.

The problems of mineral nutrition of horses depend primarily upon the facts that in their use the bones and tendons are put to such strain as to cause the many bone unsoundnesses with which all horsemen are unhappily familiar.

These unsoundnesses are ordinarily the results of maltreatment rather than malnutrition.

Under conditions of ordinarily good farm management there are no commonly obvious faults of mineral nutrition of horses. It is true that under conditions of extreme mineral nutrient deficiency horses manifest symptoms of osteoporosis, rickets, anemia and goiter, similar to those of other animals, but these conditions are comparatively rare.

Horses do not crave mineral feeds other than common salt; they are usually repelled by bone preparations, though they will sometimes take a refined steamed bone meal; but they will take and are sometimes given rock phosphate preparations—which, however, should not be fed to any animal—because of their fluorine content.

Owners of horses commonly depend on good quality of natural foods, to supply the needed mineral nutrients, and this is usually sufficient.

CATTLE.

Special concern as to the mineral nutrition of cattle (27) depends on the several-fold increase in milk production which has been accomplished by selective breeding. Cattle are rapid growing animals, and their milk is correspondingly rich in mineral nutrients. The cow, therefore, must have the supplies needed to carry on an extensive business in these substances.

The geographic mineral problem, with cattle, has to do especially with the phosphorus content of roughage, depending on the fact that there are areas of phosphorus deficient soil in many parts of the world, including several regions in the United States, but constituting a small part of the whole agricultural area.

The results of phosphorus deficiency of forage for cattle are a complication of disorders dependent upon malnutrition of the bones, depraved appetite, and generally deranged metabolism.

The administration of calcium carbonate accentuates these troubles, and the feeding of bone meal, and other phosphate foods, relieves them.

The problem of calcium content of roughage for cattle is a minor one, though there is some evidence that cattle receiving only low-calcium prairie hay for roughage may be benefited by the feeding of pulverized limestone (28).

Cattle suffer from iron deficiency in comparatively few regions, such as certain parts of Florida (29), in which the roughage is abnormally poor in iron, or in iron and copper together. The obvious method of relief is effective.

Calves do not suffer from iron deficiency, as do some other suckling animals, because they begin to eat a little hay when only a few days old—and the hay contains the needed iron.

Cattle suffer from iodine deficiency—as also do horses, sheep, and swine—in regions of low-iodine soils, foodstuffs and drinking water. This trouble appears mainly in intra-uterine development. The result, in cattle, sheep and horses, is goiter, and, in swine, also hairlessness. Iodine deficiency is easily preventable by the use of iodized salt.

Cattle may suffer from fluorine poisoning—which affects calcium metabolism, especially of the teeth (30)—but this fact seems to be practically significant at this time only in relation to the use of rock phosphate as a mineral feed.

Prominent features of the physiological mineral problem of cattle are the following:

In its practical aspects this problem relates only to the dairy cow (31–35).

It is normal for a dairy cow to be in negative calcium and phosphorus balance early in the period of lactation; later, when the mineral demands of milk production have naturally diminished, storage comes to prevail.

The annual cycle of lactation and gestation constitute the significant unit, in point of time, in this relation.

From this point of view—with ordinarily abundant milk secretion—as in average, successful production, special mineral feeds are not needed if the cow receives roughage of normal composition, in normal quantity.

Limestone and bone meal may perhaps be needed during unusually heavy milk production, or if the roughage given is restricted in quantity, or is poor in phosphorus or in calcium; but evidence as to the exact conditions which may justify the use of such feeds for dairy cows has not been established.

SHEEP.

The selective improvement of sheep has greatly increased the growth of wool by this species; and it is natural to mention sulphur metabolism in this relation, because of the high sulphur content of wool, though there is comparatively little to be said of mineral sulphur metabolism, in spite of the almost limitless ground for interest in the metabolism of organic sulphur compounds.

The nutrition of sheep doubtless requires a larger proportion of sulphur in the ration than is needed by any other animal, and it is probably more than an accident that a considerable group of brassicaceous plants, and legumes, which are rich in

sulphur, have established themselves, through the ages, as mainstays of sheep husbandry.

Sulphate and elemental sulphur have long been considered without value in the synthesis of the sulphur compounds of animal tissues, but in this relation one must not be needlessly dogmatic, because bacteria and yeasts in the alimentary tract can utilize inorganic sulphur, and the animal can digest and utilize these organisms.

Sulphur occurs in plants in many conditions and compounds—organic and inorganic—but in animals mainly as cystine, which is beta-thio-alpha-amino propionic acid, and also as methionine, which is alpha-amino gamma-methylthiol butyric acid.

According to A. T. King (36) the conversion of non-cystine sulphur into cystine, in the alimentary tract of the sheep, may depend upon bacteria which may be stimulated by iron salts in the ration, or by the sheep eating earth which is rich in iron.

Sheep suffer from forage deficiencies in calcium, phosphorus and iron—dependent on soil conditions—as do other farm animals, and likewise are benefited by the feeding of these elements in suitable inorganic compounds; but the occasions for giving mineral feeds to sheep are rare in this country—though frequent in some others.

On the borderline between organic and inorganic sulphur metabolism is the recent work, of unusual interest, by Hammett (37) and associates, which they interpret as a demonstration of the control of cell division by the naturally occurring equilibrium comprised of sulfhydryl and its partially oxidized derivatives, to which I can only allude, in passing.

SWINE.

In the mineral nutrition of swine there is no problem of soil and forage mineral deficiency, as with horses, cattle and sheep, because swine eat comparatively little roughage; but their mineral nutrition is much more likely to constitute a critical problem for this same reason—that they do eat comparatively little roughage--which is usually rich in mineral nutrients.

Other reasons for the critical situation of swine with reference to mineral nutrition are that they are raised mostly in rather close confinement, and therefore have little opportunity to gather mineral nutrients for themselves; that it is customary to

raise swine largely on low-calcium cereal feeds; and that swine develop with extreme rapidity—therefore requiring rations rich in mineral nutrients. Also they have been developed, from time to time, into physical types placing special demands upon the skeleton. In any case the heavy load of flesh which swine carry calls for strength of bones; but the refinement of skeleton which has at times been popular demands unusually dense quality of bone; at the other extreme, the so-called "big type" in the Poland China breed demanded the production of large size of bone at a rate in some cases exceeding the capacity of swine to produce normally dense bone. Also the extreme length and level back which were once popular in the Berkshire breed were found impracticable, because they placed upon the skeleton and tendons demands which they could not meet.

It is said that men will never understand the philosophy of women's hats; but men have produced some styles in pigs which suggest that men's and women's mental processes are not wholly dissimilar.

Malnutrition of the bones of swine is, under some conditions, osteoporosis—simple mineral starvation; but, under other conditions, the disorder is rickets.

The cause and the prevention of swine rickets have to do mainly with the levels of calcium and phosphorus, the proportion of calcium to phosphorus, and the vitamin D content of the ration.

The best sources of mineral nutriment for swine are leguminous roughage, skim milk, tankage, bone meal and pulverized limestone.

In addition to the skeletal problem in swine there is also, strange to say, an important problem of anemia in suckling pigs which are reared indoors, as in the winter, without contact with earth. This disorder, the nature of which was discovered by McGowan and Crichton (38) of Rowett Institute, is readily preventable by administration of iron salts, or by giving the pigs chunks of sod to chew.

POULTRY.

The following observations relating to the mineral nutrition of poultry were adapted from a statement provided by the kindly cooperation of Dr. J. E. Hunter, of the Pennsylvania Agricultural Experiment Station.

In the mineral nutrition of chickens there are two situations of special interest—rickets, and "hock disease."

Rickets, in poultry, is caused by conditions similar to those which bring about this disease in other animals, though in this relation the students of the subject have not yet reached complete agreement.

Hock disease is distinctly different from rickets. It is related to excess rather than to deficiency of mineral nutrients. Other conditions remaining the same, the percentage incidence of hock disease may be experimentally regulated in converse agreement with the bone meal content of the ration; for instance, with 2 percent of bone meal there may be an approximately 50 percent incidence, and with 4 percent bone meal a 95 percent incidence is typical.

In hock disease the tibial-metatarsal joint becomes swollen; the articular cartilage of the tibia departs slightly from its normal position; the principal tendons in this joint slip from their condiles, and the bird is permanently disabled.

Strange to say, oat hulls, or rice bran, in proper proportion in the ration, prevent hock disease in the presence of conditions which otherwise cause it; and Hunter states that he has shown that this beneficial effect cannot be attributed to the fibrous content of the supplement, or to the lowered rate of growth induced by the feeding of fibrous components. The nature of the protective substance in oat hulls and rice bran remains an interesting problem.

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Inventions and Inventors.

This volume is intended as a guide-book for those whose activities include inventive or creative procedures. Mechanical invention, chemical and electrical invention, and even psychological and biological invention are discussed. As might be surmised, the latter two discussions are highly speculative in nature. The general chapters on invention, inventors, patents, and the financing of such undertakings will be of interest to anyone with new ideas and the urge to exploit them.—L. H. S.

The Inventor and his World, by H. Stafford Hatfield. v + 269 pp. New York, E. P. Dutton and Co., 1933. $2.40.

METABOLISM AND DISEASE

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The preceding lectures of this series have been particularly instructive to one interested in metabolism primarily from the standpoint of disease. They have added to the principles which are employed in determining metabolic changes. Likewise they have cleared certain ill-understood manifestations of morbid processes. In this manner has advancing metabolic science enabled medicine better to comprehend the nature of disease. Awareness of this newer knowledge enables the surgeon, if he wishes, to escape an altogether too prevalent empiricism.

A number of diseases have been designated "idiopathic". This has resulted from a lack of understanding of the nature of the cause of the disturbance. As knowledge advances and as underlying principles are recognized, "*new*" diseases are gradually being separated from this group, isolated and defined as entities. It is obvious that such diseases are not "new". It is our discernment that is new, as an enlightened search of the older literature soon reveals. Metabolic investigation has been of especial service in this manner. Such a "new" disease is hyperparathyroidism.

HYPERPARATHYROIDISM.

Over fifty years ago a disabling, deforming disease of bone was recognized and reported (1). At first this was regarded as a peculiar form of osteomalacia, a wasting bone disease. Other instances were observed. In 1891 it was thoroughly described and given place as a disease entity by von Recklinghausen (2). It became further differentiated from other diseases presenting bone lesions. Owing to the presence of demonstrable areas of localized bone absorption, many of which contained loose fibrous tissue, it became known as osteitis fibrosa cystica. Since the bone cysts, rather than solitary, were widespread in their distribution, the modifying term "generalizata" was

added. Thus "von Recklinghausen's disease of bone" came to be recognized as involving the skeleton as a whole.

The earlier descriptions were of more advanced instances of this rare disease. These were, and are, the more readily recognized. In advanced cases the bones become demineralized. This is readily demonstrated by roentgenologic study. The local cysts may even contain areas of giant cells (3). There results an obvious weakening of the bones and an increased incidence of fractures.

In the early descriptions of the disease no mention is made of the existence of an associated parathyroid tumor. This is quite understandable, since the parathyroids were not discovered until 1880 (4). Hypoparathyroidism was only recognized in 1896 (5). The relationship to calcium metabolism was established even later (6).

Askanazy had doubtless seen an instance of the disease with an associated parathyroid adenoma (7). However, it was Erdheim's experimental studies that definitely related the parathyroids to bone disease (8). Erdheim, however, had regarded the parathyroid enlargement as a compensatory hyperplasia, and as subsequent to the bone disease, which he considered primary. Under the influence of Erdheim's hypothesis, Mandl (9) had made a homoplastic transplant of four normal parathyroids to a bed-ridden patient with von Recklinghausen's disease. The clinical symptoms, however, were definitely aggravated. Mandl then decided that the enlarged parathyroids might be the cause and not the effect of the disease. At a subsequent operation he discovered a parathyroid tumor, behind the thyroid gland. He removed it and also the transplanted parathyroids. This was followed by steady clinical improvement of the patient.

Following this striking clinical proof of the essential nature of von Recklinghausen's disease of bone, numerous similar instances were reported. About forty cases have now been studied and recorded (7, 12). Barr and Bulger (10), on the basis of clinical and experimental evidence, were the first to apply the term "hyperparathyroidism" to this rare disease.

The disease has now been reproduced experimentally (7) by administering, both acutely and chronically, Collip's (11) parathyroid extract known as "parathormone." It is even possible to produce, particularly in dogs, the characteristic bone cysts, as well as the evidence of generalized demineralization.

There are several diseases which are characterized clinically by the demineralization of bone. Compere (12a) has tabulated the principal features of seven of these. According to his studies, "hyperparathyroidism" presents five pathognomonic findings. These are: 1. A tumor of one or more parathyroids. 2. A negative calcium balance. 3. Increase in the urinary excretion of calcium. 4. Retention of sulphur. 5. The remaining parathyroids are normal grossly and microscopically. It may be added that the serum calcium is usually, although not always, elevated, and that the serum phosphorus is usually, although not always, lowered.

In this manner, too briefly sketched, has a "new" disease arisen from the "idiopathic" wasting diseases of bone. It is clearly a disorder in which there is a disturbance of calcium metabolism. The cause is a hyperfunctioning parathyroid.

IODINE METABOLISM AND THYROID DISEASE.

That a relation exists between the function of the thyroid gland and the metabolism of iodine is now undeniable. Within the past decade the nature of this relationship has been greatly clarified. Clarification has resulted, to a certain extent, from studies which have been made upon the effects of iodinization in the treatment of goiter (13). More has been learned, however, from the development of more accurate micromethods, permitting the determination of the minute amounts of iodine normally present within food, water, and air, as well as within the blood, urine and tissues (24).

The ancient use of burnt sponge in the treatment of goiter was a fortunate empiricism. Courtois discovered iodine in 1811, due to the fact that he used a lye made from seaweed, and the contained iodine corroded his copper vats. Soon after, Davy demonstrated the presence of iodine in sponges and other forms of marine life. As a natural consequence Coindet, a Swiss physician residing in Geneva, used iodine, in 1820, for the treatment of goiter (14). Since Coindet, iodine and the goiter problem have been inseparable.

Numerous subsequent investigations led to Prevost's theory in 1849. This indicated a relationship between iodine deficiency and thyroid overactivity resulting in goiter. The theory was substantiated by the work of Chatin (15). This led to the extensive use of iodized salt by the French in 1860, in an attempt to prevent the occurrence of goiter in the school children of

three departments of France (14). The ill results of this huge experiment led to abandonment of the practice. Advancing knowledge concerning the relationship between iodine and thyroid disease was consequently checked, and during the ensuing thirty-five years there arose other theories as to the nature of goiter. It was during this thirty-five year period (1860–1895) that the infection theory gained credence, doubtless due to the contemporary influence of Pasteur.

Impressed by the demonstrated interrelationship between the thyroid, goiter, and iodine, Theodore Kocher, eminent Bernese surgeon, assigned to one of his assistants the task of investigating the presence of iodine within the thyroid gland. The assistant failed. He found no iodine within the gland and thus missed making a discovery of fundamental significance. In 1895 Baumann, a biochemist, demonstrated the presence of quantities of iodine within the thyroid gland (16). As final scientific proof, Baumann actually isolated this iodine from the thyroid and demonstrated it, in its characteristic violet vapor form, in tubes (17). The significance of his contribution was quickly appreciated. Iodine in its relation to thyroid function recovered its lost prestige. The prophylactic use of iodine, properly controlled, in goiter, again became popularized. Marine and his associates (18) obtained excellent results in Ohio, as you are aware, by administering small doses of iodine. Other investigators in other countries were likewise successful (19). The lost ground was apparently recovered.

In 1919 Kendall isolated thyroxin (20). This crystalline substance, isolated from the thyroid gland, possessed certain of the physiologic properties of the dried whole gland. The isolation was repeated by Harington (21), who determined the correct molecular structure. Working from this structural formula, he succeeded in synthesizing thyroxin (22). Thyroxin, natural or synthetic, is 65 per cent iodine.

It seems obvious that the next forward step in determining more definitely the relationship between the thyroid and iodine should be an investigation of iodine metabolism. At once there arose the necessity of developing methods sufficiently sensitive, and at the same time sufficiently accurate, to determine the minute amount of iodine normally present within the ingesta, within the blood, within living tissues and within the excreta. This problem had been previously attempted by Chatin (15); however, his work had been doubted.

The iodine content of the thyroid gland itself was readily determined by the older, coarser methods, owing to the relatively high iodine concentration (23). This did not hold for the blood. Davy had originally separated and determined the iodine present in sponges by first ashing the organic material. The principle of his method, with various modifications, has since been widely followed. For decades, numerous methods had been devised for the determination of the iodine content of food, water, and air (24). None of these, however, was sufficiently delicate even to detect the minute amount of iodine normally present within the ordinary quantities of blood used for analyses.

During the first decade of this century advancing goiter research created an acute demand for an adequate micromethod. In 1922 the Swiss Goiter Commission, consequently, requested von Fellenberg, chemist of the Bureau of Hygiene, to develop such a method. He devised, perfected, and eventually synthesized the known existing quantitative procedures into an adequate micromethod for the determination of iodine (24). This will determine one ten-thousandth of a milligramme, 0.0001 mg. Two of von Fellenberg's pupils, Sturm (25) and Lunde (26), soon applied this method to a study of the blood, particularly in patients with thyroid disease. Thus was opened the way for a new approach to our conception of thyroid function in health and disease.

Following Baumann's discovery, numerous investigators had determined the iodine content of normal and of goitrous thyroid glands (27). Kendall and others (28) had observed a seasonal variation in the iodine content of the thyroid. This was lowest in the winter and spring and highest in the summer and autumn. These findings, by the way, correspond to what we now know of the associated blood iodine level (Sturm).

In investigating the intermediary metabolism of iodine, it was inevitable that attention should be directed to the blood. In 1899 Gley and Bourcet (29) unable to demonstrate iodine in 100 to 200 cc. samples, used a liter of dog's carotid blood for their analyses. They thus were able to demonstrate the presence of iodine in mammalian blood. They regarded it as a normal constituent of the blood stream. This was subsequently confirmed by some but denied by others (30). It was a matter of method. The earlier analyses were quantitatively inaccurate as judged by the results of modern micromethods.

The "*first modern figure*" for the blood iodine is that of Kendall, 13 gamma per cent for ox blood (31). The normal iodine content of blood is so minute that the term *gamma* is used to designate its unit. One gamma equals one one-thousandth of a milligramme, 0.001 mg. The normal iodine content of human blood in the Chicago district we have found to be about 12 gamma per cent, or .012 milligrammes in 100 cc. of blood (32). Attempts have been made at separating the iodine of the blood into an alcohol insoluble "organic" portion or one presumably in protein combination, and an "inorganic" portion or one which is alcohol soluble (33).

From an extensive investigation which our group has made during the past four years, it is apparent that a definite interrelationship exists between thyroid activity and the blood iodine niveau (34). In this our findings confirm those of Sturm and of Lunde. I wish to present, at this time, certain of the results which we have obtained in determining the blood iodine level of normal individuals and of over 150 patients with various diseases of the thyroid gland. These analyses have all been made by Dr. Chester Davis, Dr. Versa Cole, and Mr. Francis Phillips. Dr. Davis spent, at the commencement of our work, five months experimenting with and developing the method before it was applied to the blood of patients. The method he developed (35) was essentially that of von Fellenberg (24). Mr. Phillips has made important additions. It is possible to determine one ten-thousandth of a milligramme of iodine. Contrary to general opinion, the technical difficulties encountered concern mainly iodine loss rather than contamination.

Before considering the blood iodine data, several facts should first be recalled and emphasized. The diffusely enlarged, hyperplastic gland of untreated Grave's disease is ordinarily iodine-poor (27). It contains, as a rule, less iodine than the normal thyroid gland. Little or no colloid is present (36). This represents the overfunctioning thyroid. The amount of iodine within the gland increases from three to ten times following the customary preoperative administration of iodine (Lunde and Holst). This is accompanied by filling of the alveoli with colloid.

The normal blood iodine ranges from 8.5 to 16.2 gamma per cent, averaging 12 gamma per cent (37). This statement is based upon 34 determinations made upon twenty-eight

individuals with no evidence of thyroid dysfunction. Eight determinations made on six normal persons ranged between 8.9 and 13.8 gamma per cent, averaging 12 gamma per cent. It is necessary to determine the blood iodine of women in the intermenstrual period, since there is a rise in the blood iodine during the onset of menstruation (38). Thirteen determinations made on ten hospital patients on ordinary hospital diets revealed a range of from 8.5 to 13.4 gamma per cent and an

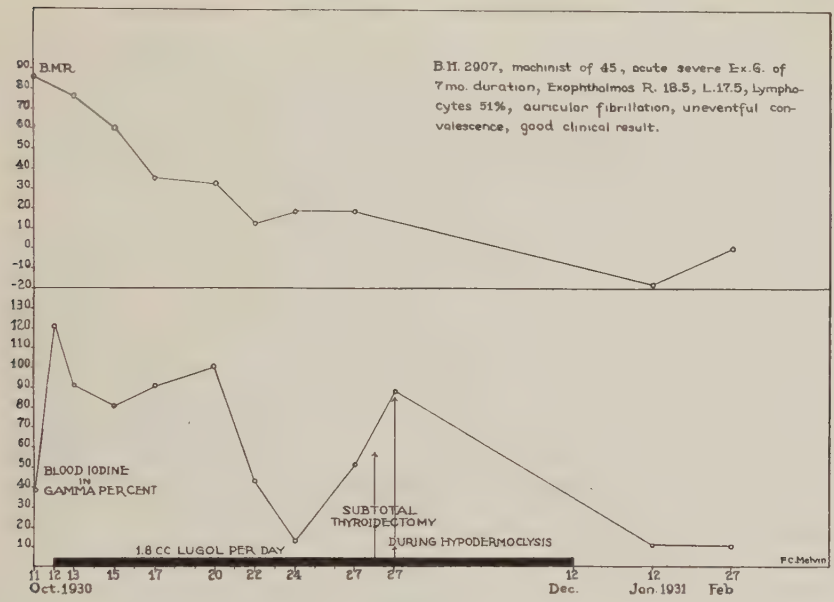
B.M.R.
B.H. 2907, machinist of 45, acute severe Ex.G. of 7mo. duration, Exophthalmos R. 18.5, L.17.5, Lymphocytes 51%, auricular fibrillation, uneventful convalescence, good clinical result.
BLOOD IODINE IN GAMMA PER CENT
SUBTOTAL THYROIDECTOMY
DURING HYPODERMOCLYSIS
1.8 CC LUGOL PER DAY
F.C.Melvin
Oct. 1930
Dec.
Jan. 1931
Feb

FIGURE 1.

THE BLOOD IODINE IN EXOPHTHALMIC GOITER.

The blood iodine is elevated previous to medication (normal = 12 gamma per cent). It rises upon lugolization. It is high during iodine administration by hypodermoclysis. It falls to a low normal level subsequent to subtotal thyroidectomy and the cessation of lugolization. The elevated B. M. R. falls upon lugolization. It remains low after an adequate thyroidectomy.

average of 12.3 gamma per cent. Iodized salt was not used in the hospital diet. No significant change was noted in the blood iodine of patients with cancer. Thirteen determinations made on twelve patients in the Out-Patient Department gave a range of from 8.5 to 16.2 gamma per cent, averaging 11.4 gamma per cent.

In acute hyperthyroidism, known clinically as Grave's disease, there is a marked elevation (Figure 1) of the blood

iodine (39). Sixteen determinations made on eleven of these unmedicated patients revealed a range of from 16.9 to 40.1 gamma per cent, or an average of 27.1 gamma per cent. The average basal metabolic rate in these eleven patients was plus 50. No direct correlation was observed between the height of the basal metabolic rate and the blood iodine level. We have thus far made no attempt to fractionate this high blood iodine into "organic" and "inorganic" portions.

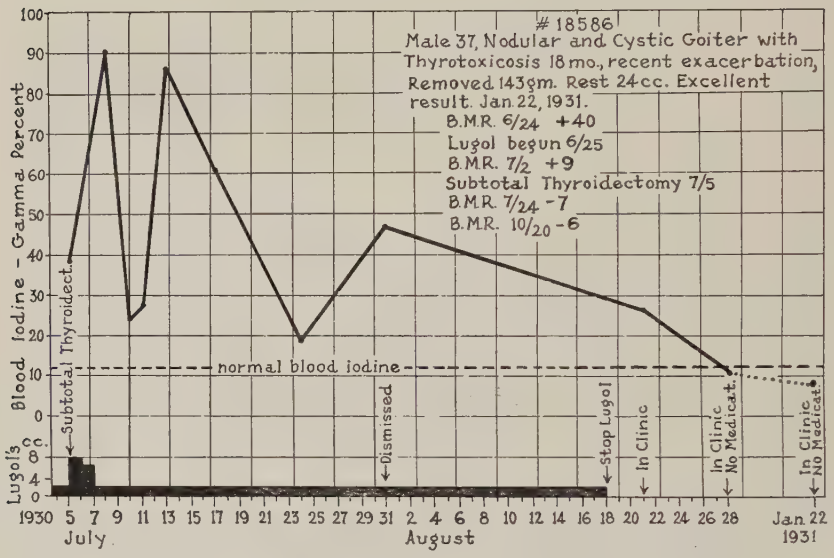
#18586
Male 37, Nodular and Cystic Goiter with Thyrotoxicosis 18 mo., recent exacerbation, Removed 143 gm. Rest 24 cc. Excellent result. Jan. 22, 1931.
B.M.R. 6/24 +40
Lugol begun 6/25
B.M.R. 7/2 +9
Subtotal Thyroidectomy 7/5
B.M.R. 7/24 -7
B.M.R. 10/20 -6
Blood Iodine - Gamma Percent
normal blood iodine
Subtotal Thyroidect.
Dismissed
Stop Lugol
In Clinic
In Clinic No Medicat.
In Clinic No Medicat.
Lugol's cc.
1930 July August 1931 Jan. 22

FIGURE 2.

THE BLOOD IODINE IN TOXIC NODULAR GOITER.

Following subtotal thyroidectomy the blood iodine remains elevated so long as lugolization is continued. It falls to a low normal level after adequate thyroidectomy and cessation of iodine medication.

After an adequate thyroidectomy, with subsequent relief of the symptoms of the hyperthyroidism, the elevated blood iodine falls to a low normal range (Figure 1). Fifteen determinations made on twelve operated patients revealed a range of from 8.7 to 14.3 gamma per cent, or an average of 10.4 gamma per cent. The basal metabolic rate in these patients averaged minus 6.

In the more chronic and less severe form of hyperthyroidism occurring in patients with nodular goiters, the blood iodine is also elevated, (Figure 3). Eleven determinations on eleven of these patients revealed a range of from 13.1 to 38.1 gamma per cent, averaging 22 gamma per cent. The average basal

metabolic rate was plus 26. Thus chronic hyperthyroidism is accompanied by a lesser elevation of the blood iodine, as well as by a lesser elevation of the basal metabolic rate. Likewise,

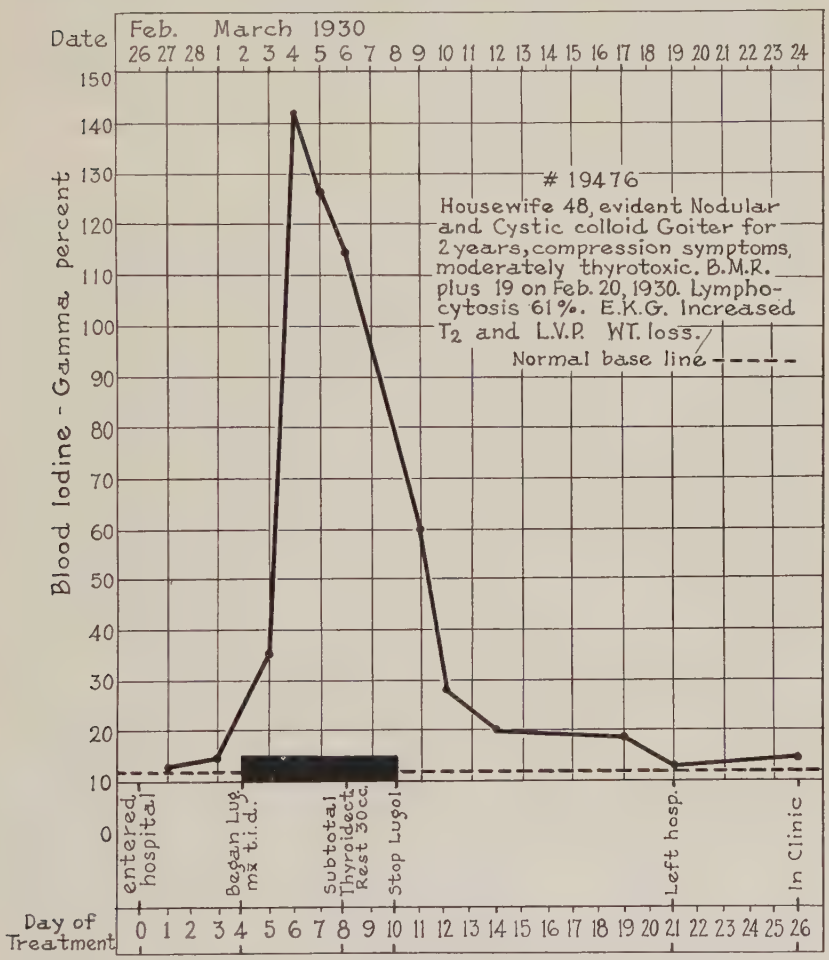
Date
Feb. March 1930
26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Blood Iodine - Gamma percent
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
19476
Housewife 48, evident Nodular and Cystic colloid Goiter for 2 years, compression symptoms, moderately thyrotoxic. B.M.R. plus 19 on Feb. 20, 1930. Lymphocytosis 61%. E.K.G. Increased T2 and L.V.P. WT. loss.
Normal base line
entered hospital
Began Lug mx t.i.d.
Subtotal Thyroidect. Rest 30cc.
Stop Lugol
Left hosp.
In Clinic
Day of Treatment
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

FIGURE 3.

THE BLOOD IODINE IN MODERATELY TOXIC NODULAR GOITER.

The premedication level is above normal. A marked rise occurs upon lugolization and continues so long as iodine is administered. There is still some elevation two weeks after thyroidectomy and the cessation of lugolization.

in toxic nodular goiter, no direct correlation was observed between the blood iodine and the elevated basal metabolic rate. After these patients have submitted to an adequate

thyroidectomy, with the subsequent symptomatic relief, the blood iodine falls to a low normal level, (Figure 2). Twenty-three determinations, made on twelve of these patients, revealed a range of from 5.5 to 16.9 gamma per cent, averaging 10.4 gamma per cent. The average basal metabolic rate in these twelve patients was minus 4. It is thus apparent, that when hyperthyroidism is controlled by adequate removal of the over-functioning gland, the elevated blood iodine falls to a low normal level. This is shown in Figures 1 and 2.

If the thyroidectomy is inadequate, the blood iodine does not fall to a low level. It also remains elevated between the stages of a two stage lobectomy. In four determinations made on four of these patients, the range was from 19.4 to 32 gamma per cent, averaging 26.1 gamma per cent. The average basal metabolic rate in these patients was plus 31.

Nontoxic nodular goiter, presenting no symptoms of hyperthyroidism, is accompanied by a low normal range of the blood iodine. In eighteen determinations made on ten of these patients, the range was from 6.3 to 12.7 gamma per cent, averaging 10.4 gamma per cent. The average basal metabolic rate in these ten patients was minus 4. Following partial thyroidectomy for nontoxic, nodular goiter, little eventual change occurs in the elevation of the blood iodine. This is shown in Figure 3. In these patients more of the thyroid is left behind than in those patients with toxic goiter.

The blood iodine is variable in its elevation in various other diseases. It is definitely elevated in lymphatic leukemia. It is to be remembered that in this disease there is an elevation of the basal metabolic rate, of the lymphocyte count of the blood, and that beneficial effect ensues after iodine treatment. We may now add that the blood iodine is elevated (40). This striking parallelism between hyperthyroidism and lymphatic leukemia is worthy of further investigation.

In those patients who, normal metabolically, develop exophthalmos subsequent to an adequate thyroidectomy, the blood iodine is normal (40). In unmedicated hypothyroidism the blood iodine is low (40). In eleven determinations made on eight of these patients, the range was from 7.2 to 11 gamma per cent, averaging 9.4 gamma per cent. The average basal metabolic rate was minus 16. When these patients are given desiccated thyroid by mouth, there occurs, along with the clinical improvement, a rise in the iodine content of the blood.

It is difficult to dissociate this rise from the amount of iodine administered in the desiccated thyroid. Immediately following administration, there ensues a sharp rise in the blood iodine. This precedes that of the basal metabolism. In patients with treated hypothyroidism the blood iodine is elevated. This elevation is greater at first and becomes stabilized at a high normal level after continued desiccated thyroid administration.

Members of the same family, with varying states of thyroid function due to disease, present striking variations of the blood iodine. In one family investigated, the blood iodine of the mother was high. She had toxic nodular goiter. The blood iodine was normal in one son with no evidence of thyroid disease and low in another son and daughter who had diffuse goiters with hypothyroidism.

Immediately following a thyroidectomy, there ensues a fall in the blood iodine. It remains to be seen whether this is directly the result of the removal of an excess amount of the secreting thyroid tissue.

In children with acute hyperthyroidism, the blood iodine is definitely elevated (41). It responds to iodine medication and thyroidectomy as does the blood iodine in adults. Subsequent to thyroidectomy for toxic goiter, the blood iodine remains high so long as iodine medication is continued. Upon cessation of iodine medication, however, it falls to a low normal level, provided an adequate thyroidectomy has been made. This effect is presented in Figure 1.

Judging from an extensive clinical study of the blood iodine in patients with varying states of thyroid activity, there is a definite relationship between the level of the blood iodine and thyroid function (40).

DEMINERALIZATION.

The significance of other blood electrolytes in relation to disease is also deserving of our attention. Changes in the normal concentration of certain ions, or fluctuations in the balance between others, are associated with certain morbid processes occurring spontaneously or produced experimentally. Subsequent to surgical operations, during which ether is used as the anesthetic, there ensue extensive changes in the calcium-potassium ratio of the blood (42). These are accompanied by a period of post-operative lethargy, and a marked tissue thirst. Calcium loss, sufficient to result in osteoporosis, may ensue

following the continuous loss of bile subsequent to the establishment of a chronic biliary fistula (43). The blood bromine is now receiving clinical attention (55).

The chloride of the blood, since it is the most prevalent ion and the more readily determined by various analytical methods, has been particularly studied. An extensive literature has already accumulated. The hypochloremia subsequent to upper intestinal obstruction has been frequently confirmed (44). A similar lowering of the blood chloride is associated with the fatal effect of the total loss of gastric juice (45). Orr and Haden (46) report that the blood chloride is also lowered in dogs with fatal experimental peritonitis. We have observed a low blood chloride accompanying a strangulated hernia of the omentum, and in cases of acute diffuse peritonitis. The blood chloride is low despite the blood concentration of the so-called toxemia following extensive superficial burns (47). The fluid within the blisters has a chloride concentration higher than that of the blood plasma. The symptoms of heat cramps, called also miner's cramp, stoker's cramp, or fireman's cramp, are initiated by a marked loss of perspired chloride and are prevented by drinking saline or relieved by the administration of salt solutions (48). The symptoms of "water intoxication" as studied and described by Rowntree (49) are relieved by saline intravenously. In pneumonia the serum chloride is lowered and the urinary excretion of chloride decreases or even fails. In view of these and of other similar clinical findings, it appears that the disturbance in chloride metabolism associated with these morbid states is possibly of more than subordinate importance.

Various crystalloids may be readily dialyzed away from the circulating blood through celloidin tubes inserted in the course of the blood stream. This process, called *vividiffusion*, was devised by Abel, Rowntree, and Turner (51). Dialysis also readily occurs through the peritoneum. Cohnheim (52) found that chloride soon appears in glucose solutions injected intraperitoneally in rabbits. This is likewise true when varying concentrations of cane sugar are employed (53). When distilled water is injected intraperitoneally, a higher per cent of chloride, up to 0.63 per cent, is deviated from the blood stream and the blood chloride falls (54). The intraperitoneal water soon becomes isosmotic and obtains up to 1.5 per cent of albuminous substance as well as numerous cells. Achloride electrolytes, as

well as organic crystalloids, are likewise dialyzed from the blood stream (54). Consequently, it has been possible, experimentally, to demineralize rabbits by perfusing distilled water through the peritoneal cavity (50, 60).

The transperitoneal perfusion of distilled water at body temperature at the rate of 500 cc. per hour results, within an hour, in an increase in the respiratory rate and the appearance of localized fibrillary twitchings of the muscles (50). These become more generalized, and are followed by clonic and tonic convulsions of increasing severity. Spasmodic contractions of the diaphragm occur, and the animals ordinarily die after from two to five hours of perfusion. The blood chloride falls. The carbon dioxide combining power of the plasma falls. There is a moderate rise in the N. P. N., and a slight elevation of the urea. The secretion of urine soon diminishes and finally completely ceases. The development of the symptoms and of the lethal effect are not due to hypoglycemia since addition of 0.12 per cent glucose to the perfusion water in other experiments was without preventive effect.

Transperitoneal perfusion of Ringer's solution, or of isosmotic pure sodium chloride, with or without glucose, resulted in the development of no muscular twitchings or convulsions. These animals were alive and in good condition when the experiments were terminated at the end of eight to ten hours. The blood chloride was slightly elevated. There was a continuous secretion of urine, or even a moderate diuresis. In one of the sodium chloride perfusion experiments the serum calcium fell to 2.9 mg. per 100 cc. of blood without evidence of tetany.

The transperitoneal perfusion of 4.2 per cent glucose resulted in increased respirations, muscular twitchings and tremors, convulsions and eventually death in from two and one-half to seven hours. The terminal blood chloride was low. There was an enormous rise in the blood sugar. Perfusion of 0.45 per cent sodium chloride and 2.1 per cent glucose had no such lethal effect and the animals lived.

The perfusion of Ringer's solution with glucose, but *without sodium chloride*, resulted in increased respirations, muscular twitchings and convulsions and eventual death. The blood chloride fell. The secretion of the urine diminished and then ceased.

An adequate amount of sodium chloride was eventually supplied to the blood stream during the transperitoneal per-

fusion with distilled water. This was accomplished by the timed intravenous injection of 2.5 per cent pure sodium chloride by means of the Woodyatt pump. It was found best to inject a 2.5 per cent solution of pure sodium chloride at the rate of 1 cc. per minute into one of the cannulated jugular veins. When too concentrated solutions were used, e. g., 9.0 per cent, thrombosis occurred locally and was even followed by pulmonary embolism. If the solution is too dilute, sodium chloride is not supplied as rapidly as it is dialyzed away; also, too much water must be simultaneously injected. The continuance of renal secretion is apparently an important factor in the success of these experiments.

By this method it was possible to keep the animals alive. One animal lived seventeen and one-half hours, more than five times as long as during the transperitoneal perfusion of distilled water alone. Occasional mild muscular fibrillation was noted, but no severe tremors or no convulsions. A marked diuresis was observed. There was a slight fall in the whole blood chloride. There was no tetany although the serum calcium fell to 3.8 mg. per 100 cc.

In succeeding experiments the combined intravenous infusion and transperitoneal perfusion were similarly maintained for twelve hours without the development of notable symptoms. At the end of that time the intravenous injection of pure sodium chloride was stopped, but the transperitoneal perfusion of distilled water with glucose continued. Muscular twitchings then developed, became more severe and finally convulsions occurred and death followed after five hours of transperitoneal perfusion alone.

It is difficult to make any greatly inclusive judgments as to the bearing of these experiments upon our daily clinical problems. So much is unknown, particularly regarding the associated physico-chemical blood changes. Consequently, it is with some hesitation that I shall consider their significance in four conditions which we encounter in the post-operative management of our surgical cases: dehydration, anuria, tetany, and hypochloremia.

Dehydration is frequently evoked to explain the results of the extensive loss of fluid by profuse sweating, in extensive burns, by vomiting, diarrhea, or through the various gastro-intestinal fistulae. On the other hand, we must be careful to remember that all these fluids have a considerable mineral

content which likewise is simultaneously lost to the organism. In replacing this fluid loss, we do not administer distilled water, but rather salt solutions.

During the past few years, we have analyzed chemically the body fluids found in various morbid conditions such as ascites, hydrothorax, pericardial effusion, hydrocele, blisters following burns, synovial effusions, effusions in bursae, ovarian cysts and tendon sheaths (56). Nearly all of these have a high mineral content. The copious intestinal fluid found in the paralytic ileus accompanying peritonitis has a high mineral content, likewise the intestinal fluid above a mechanical obstruction or that draining from a jejunostomy. The loss of these fluids is thus accompanied by a considerable loss of minerals. As a consequence together with the dehydration, demineralization is to be considered. These experiments demonstrate certain of the effects of demineralization as produced by peritoneal dialysis. Consequently, in our replacement therapy, we should be aware of the demineralization associated with the loss of body fluids.

The treatment of the recurrent ascites of portal cirrhosis by biweekly paracentesis of the abdomen is of interest in this connection. So much as ten liters of fluid may be obtained at each tapping. It is not generally recognized that this fluid has a high mineral content. We have determined this as about 0.7 per cent (56). Thus every two weeks over 70 grammes of minerals are removed from the patient, as well as protein and other crystalloids.

The anuria associated with intestinal obstruction is commonly thought to be due to a "toxemia" which in some manner depresses the function of the kidney. Our experiments show, when the organism is being demineralized by the transperitoneal perfusion of distilled water, that the secretion of urine soon decreases and finally ceases. That this is not due to some toxic action upon the kidney is shown by the fact that the kidney resumes function upon the simultaneous administration of timed adequate salt solution intravenously (57). In a study of the mechanism of diuresis we found it possible to inhibit or even block the normal action of a theophyllin diuretic administered intramuscularly, by simultaneously injecting distilled water intraperitoneally (58). This intraperitoneal distilled water caused a rapid dialysis of salts from the blood stream and tissues into the artificial transudate. The minerals were thus

deviated. When the artificial transudate became isosmotic, a diuresis ensued. Diuresis is dependent to a great extent upon the state of the tissues (58). Changes in this state due to mineral loss may readily affect the function of the kidney. In this manner we would regard certain cases of postoperative anuria as due to a depletion of blood or tissue electrolytes rather than as resulting from some "toxic" or "reflex" (59) effect.

Various forms of tetany have been recognized clinically. Most commonly it is regarded as being associated with hypoparathyroidism and a lowered blood calcium. There has been a tendency to explain the various types of tetany upon this basis. We have produced tetany in animals by perfusing distilled water through the peritoneal cavity (50). This tetany may be relieved by the simultaneous intravenous injection of adequate pure sodium chloride. When pure physiologic saline is perfused through the peritoneal cavity, tetany does not usually occur even though the blood calcium falls (50). It would seem from our experiments and from a study of certain morbid states, that certain forms of tetany are associated with demineralization and particularly with an extensive loss of chloride.

The frequent occurrence of lowered blood chloride in various morbid conditions and the relief of the symptomatology in certain of these conditions by the administration of sodium chloride by mouth, by hypodermoclysis, or intravenously, indicate the significance of hypochloremia. It is difficult properly to evaluate with the evidence at hand the complex physico-chemical changes which occur in the blood and tissues of the animals which we have perfused with distilled water or various other solutions. The evidence, however, which we have, both from the literature and from these briefly presented experiments leads us to reemphasize the significance of the minerals to life and a state of well being.

The evidence for the significance of calcium and its relation to parathyroid function is convincing. Likewise the interrelation between thyroid function and iodine is inescapable. The specificity of chloride is not so clear.

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